مركز الشامل للخدمات الطلابية

Biochemistry

I

مركز الفرقان للخدمات الطلابية

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COENZYMES & VITAMINS

Vitamins are organic compounds required by the body in trace amounts to perform specific cellular functions, for example, many of the water-soluble vitamins are precursors of coenzymes for the enzymes of intermediary metabolism. In contrast to the water-soluble vitamins, only one fat soluble vitamin (vitamin K) has a coenzyme function. Nine vitamins (folic acid, cobalamin, ascorbic acid, pyridoxine, thiamine, niacin, riboflavin, biotin, and pantothenic acid) are classified as water-soluble, whereas four vitamins (vitamins A, D, E, and K) are termed fat-soluble. Vitamins cannot be synthesized in adequate quantities by humans and, therefore, must be supplied by the diet.

The water-soluble vitamins are not toxic, and the amounts stored in the body are usually small. When ingested in excess of the body's need, they are readily excreted in the urine.

The fat-soluble vitamins are released, absorbed, and transported with the fat of the diet. They are not readily excreted in the urine, and significant quantities are stored in the liver and adipose tissue. In fact, consumption of vitamins A and D in excess of the recommended dietary allowances can lead to accumulation of toxic quantities of these compounds.

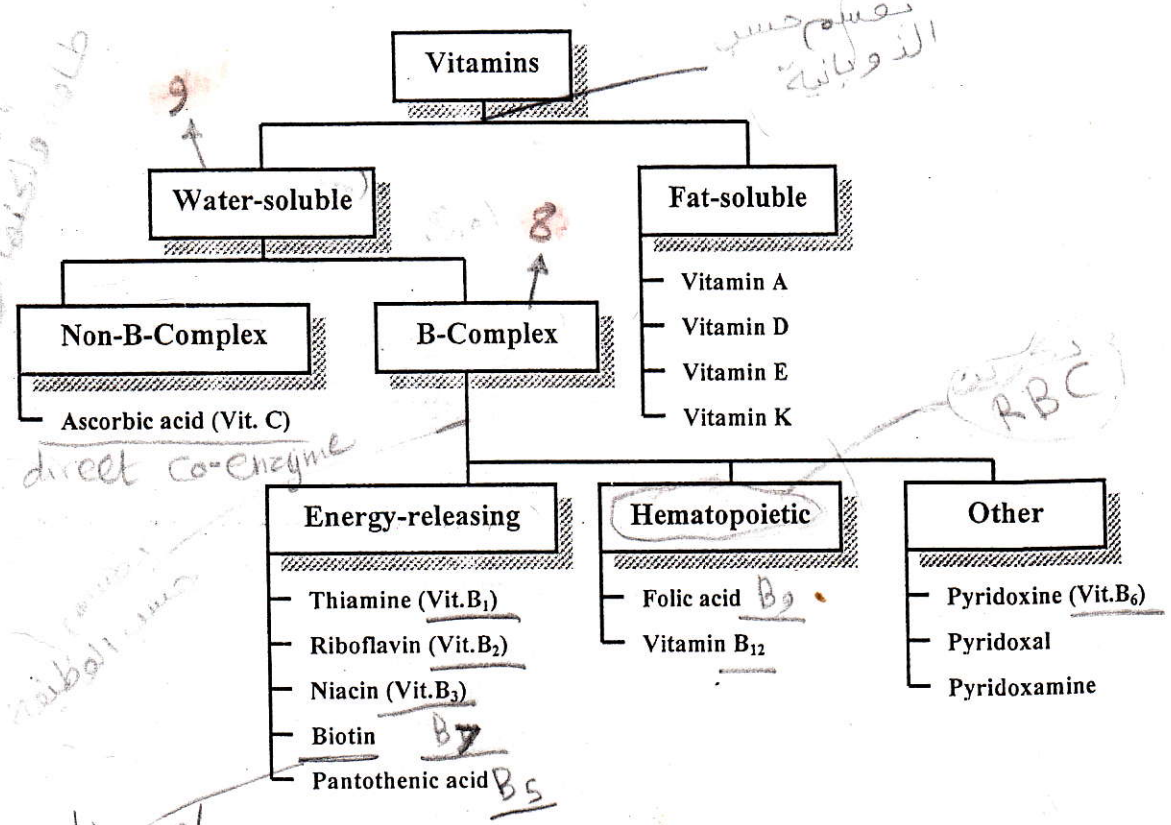


Figure: Classification of the vitamin.

Thiamine pyrophosphate (TPP):

Thiamine pyrophosphate is the biologically active of Thiamine (vitamin B_1). TPP serves as a coenzyme in the formation or degradation of α-ketols by transketolase, and in the oxidative decarboxylation of α-keto acids TPP, also appears to play an important role in the transmission of nerve impulses. The synthesis of acetylcholine requires the presence of TPP.

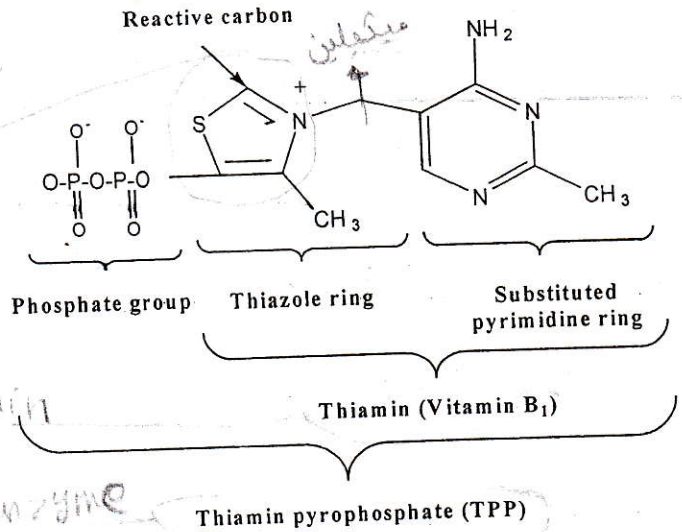


Figure: Thiamine pyrophosphate.

The common site of action is C_2 of the thiazole ring.

Pork, whole grains (outer layers), legumes are richest sources of thiamine.

A. Clinical indications for thiamine

The oxidative decarboxylation of pyruvate and α-ketoglutarate, which plays a key role in energy metabolism of most cells, is particularly important in tissues of the nervous system. In thiamine deficiency, the activity of these two dehydrogenase-catalyzed reactions is decreased, resulting in a decreased production of ATP and, thus, impaired cellular function.

1. Beriberi: This is a severe thiamine-deficiency syndrome found in areas where polished rice is the major component of the diet. Signs of infantile beriberi include tachycardia, vomiting, convulsions, and, if not treated, death. The deficiency syndrome can have a rapid onset in nursing infants whose mothers are deficient in thiamine. Adult beriberi is characterized by dry skin, irritability, disordered thinking, and progressive paralysis.

2. Wernicke-Korsakoff syndrome: In the United States, thiamine deficiency, which is seen primarily in association with chronic alcoholism, is due to dietary insufficiency or impaired intestinal absorption of the vitamin. Some alcoholics develop Wernicke-Korsakoff syndrome—a thiamine deficiency state characterized by apathy, loss of memory, ataxia, and a rhythmic to-and-fro motion of the eyeballs (nystagmus). The neurologic consequences of Wernicke's syndrome are treatable with thiamine supplementation.

Flavin-coenzymes (FMN & FAD):

Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are the two biologically active forms (coenzymes) of riboflavin (vitamin B_2). FAD is formed by the transfer of an AMP moiety from ATP to FMN.

FMN = vit. B_2 + phosphate group.

FAD = FMN + AMP.

FMN and FAD are each capable of reversibly accepting two hydrogen atoms, forming $FMNH_2$ or $FADH_2$.

FMN and FAD are involved as coenzymes in oxidation-reduction reactions.

Milk, eggs, liver and green leafy vegetables are good sources of riboflavin.

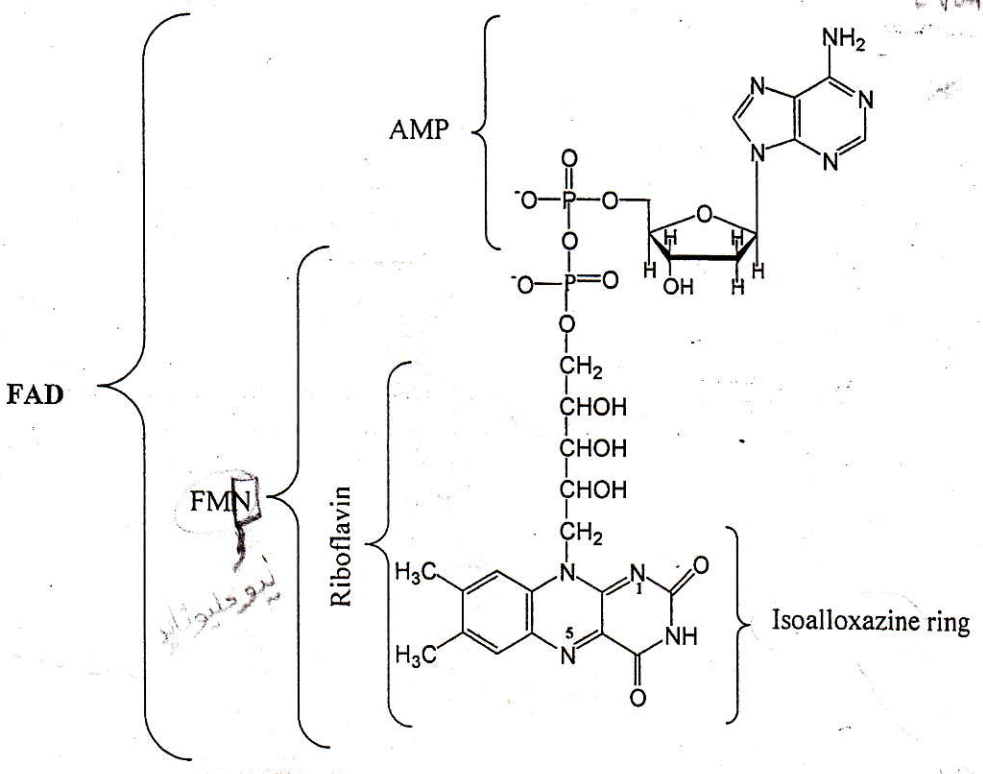


Figure: Structure of flavofavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and riboflavin

Deficiency symptoms include dermatitis, cheilosis (fissuring at the corners of the mouth), and glossitis (the tongue appearing smooth and purplish).

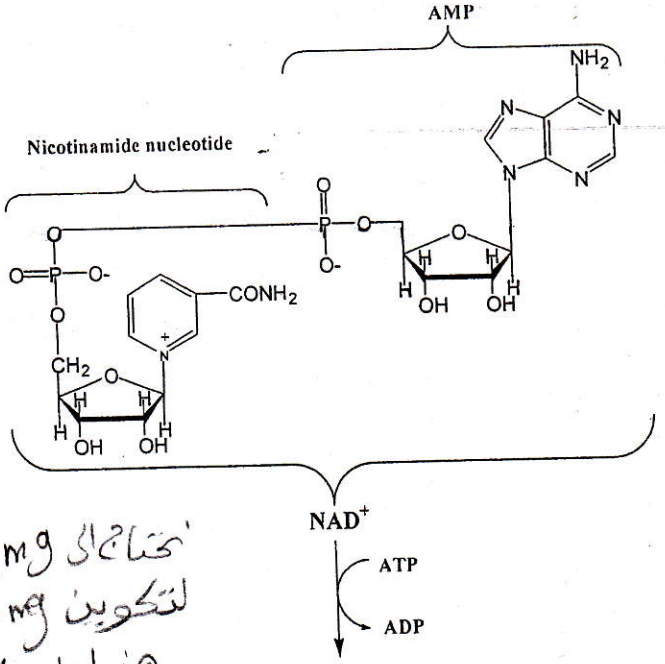
Pyridine nucleotides (NAD^+ & $NADP^+$):

Nicotinamide adenine dinucleotide (NAD^+) is a biologically active coenzyme composed of two parts:

1. Nicotinamide nucleotide. 2. Adenine nucleotide (AMP).

Nicotinamide nucleotide is derived from the vitamin niacin (or nicotinic acid).

Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is another biologically active coenzyme.



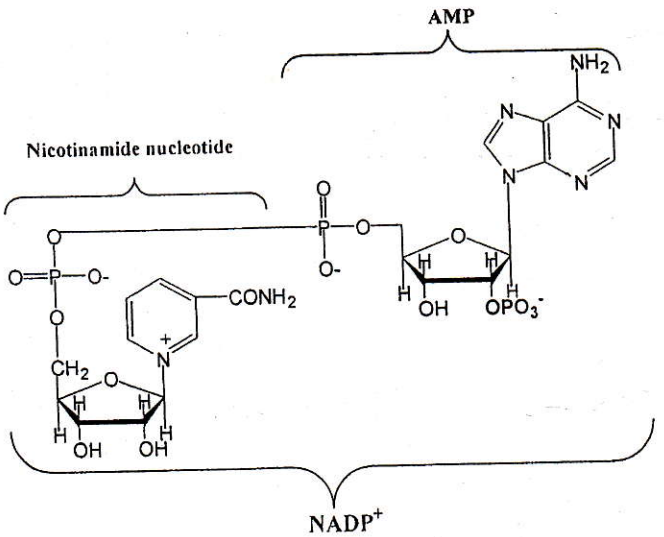


Figure: Structure of NAD^+ and $NADP^+$.

NAD^+ and $NADP^+$ serve as coenzymes in oxidation-reduction reactions in which the coenzyme undergoes reduction of the pyridine ring by accepting a hydride ion (H^-). The reduced forms of NAD^+ and $NADP^+$ are NADH and NADPH, respectively.

Bran of grain and cereals, liver, meat, fish, milk are good sources for niacin. [Note: Corn is low in both niacin and tryptophan. Corn-based diets can cause pellagra.]

Clinical indications for niacin

1. Deficiency of niacin: A deficiency of niacin causes pellagra, a disease involving the skin, gastrointestinal tract, and CNS. The symptoms of pellagra progress through the three Ds: dermatitis, diarrhea, dementia—and, if untreated, death.

2. Treatment of hyperlipidemia: Niacin (at doses of 1.5 g/day or 100 times the Recommended Dietary Allowance or RDA) strongly inhibits lipolysis in adipose tissue—the primary producer of circulating free fatty acids. The liver normally uses these circulating fatty acids as a major precursor for triacylglycerol synthesis. Thus, niacin causes a decrease in liver triacylglycerol synthesis, which is required for very-low-density lipoprotein (VLDL) production. Low-density lipoprotein (LDL, the cholesterol rich lipoprotein) is derived from VLDL in the plasma. Thus, both plasma tri acylglycerol (in VLDL) and cholesterol (in VLDL and LDL) are lowered. Therefore, niacin is particularly useful in the treatment of Type IIB hyperlipoproteinemia, in which both VLDL and LDL are elevated. [Note: Niacin raises HDL levels.]

Biotin:

Biotin (vit. B_7) is covalently bound to the ε-amino group of lysine residue of biotin-dependent enzyme. The coenzyme form is called as biocytin. It is a coenzyme in carboxylation reactions, in which it serves as a carrier of activated carbon dioxide (CO_2).

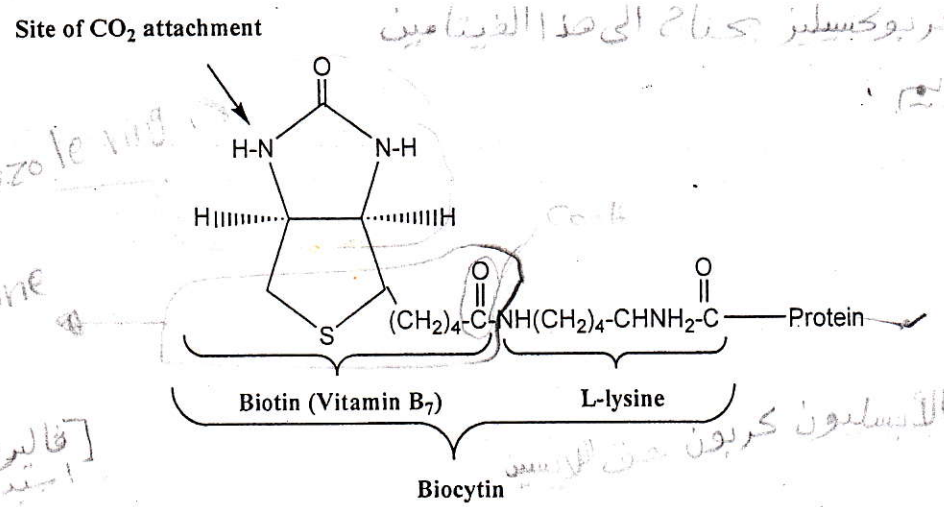


Figure: Structure of biotin and biocytin covalently bound to a lysyl-residue of a biotin-dependent enzyme.

Biotin is present in almost all foods, particularly liver, milk, and egg yolk. Bacterial flora in human intestine can synthesize the vitamin in a sufficient amount.

Biotin deficiency does not occur naturally. However, the addition of large amounts of row-egg white to the diet as a source of protein induces symptoms of biotin deficiency, namely dermatitis, glossitis, loss of appetite, and nausea. Raw egg white contains a glycoprotein, **avidin**, which tightly binds biotin and prevents its absorption from the intestine. Thus, inclusion of an occasional raw egg in the diet does not lead to biotin deficiency, although eating raw eggs is generally not recommended due to the possibility of salmonella infection.

Coenzyme A (CoA~SH):

Coenzyme A is the biological active form of the vitamin pantothenic acid. It functions in the transfer of acyl groups. Coenzyme A contains a thiol (-SH) group that carries acyl compounds as activated thiol esters.

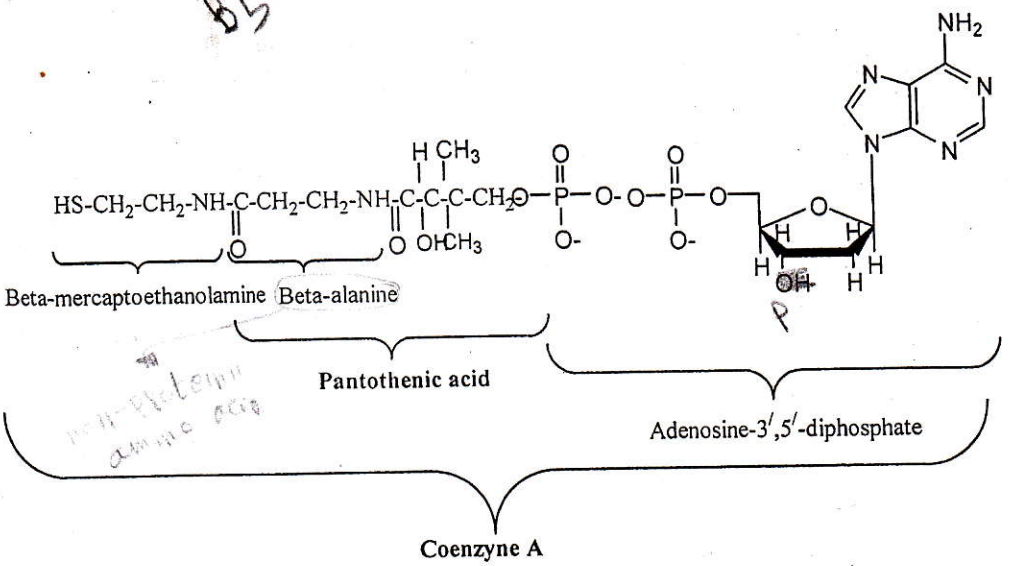


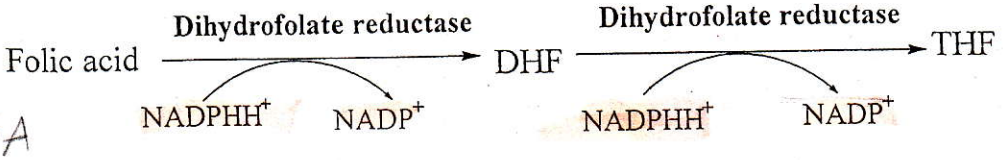
Figure: Structure of Coenzyme A.

Examples of such structures are succinyl CoA, fatty acyl CoA, and acetyl CoA. In the form of acetyl CoA, it participates in a number of important metabolic reactions.

Eggs, liver, an d yeast are the most important sources of pantothenic acid.

Folic acid:

Folic acid (or folate) is the vitamin. The biologically active (coenzyme) form of folic acid is tetrahydrofolic acid (THF), which is produced by the two-step reduction of folate by **dihydrofolate reductase**.



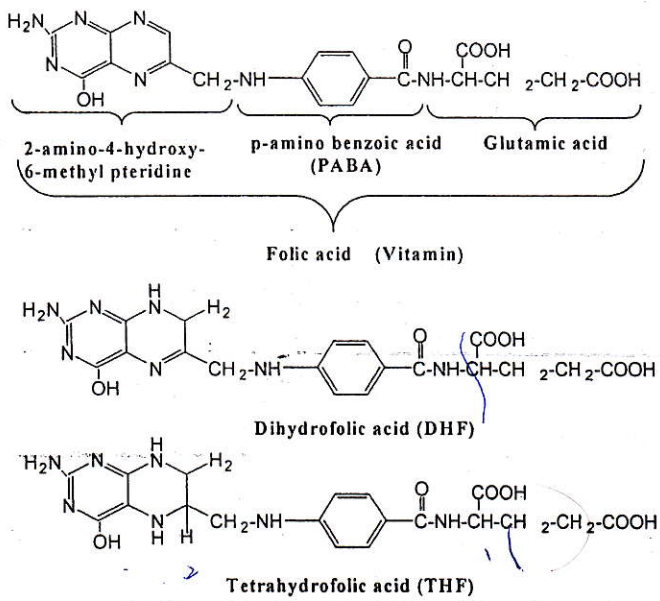
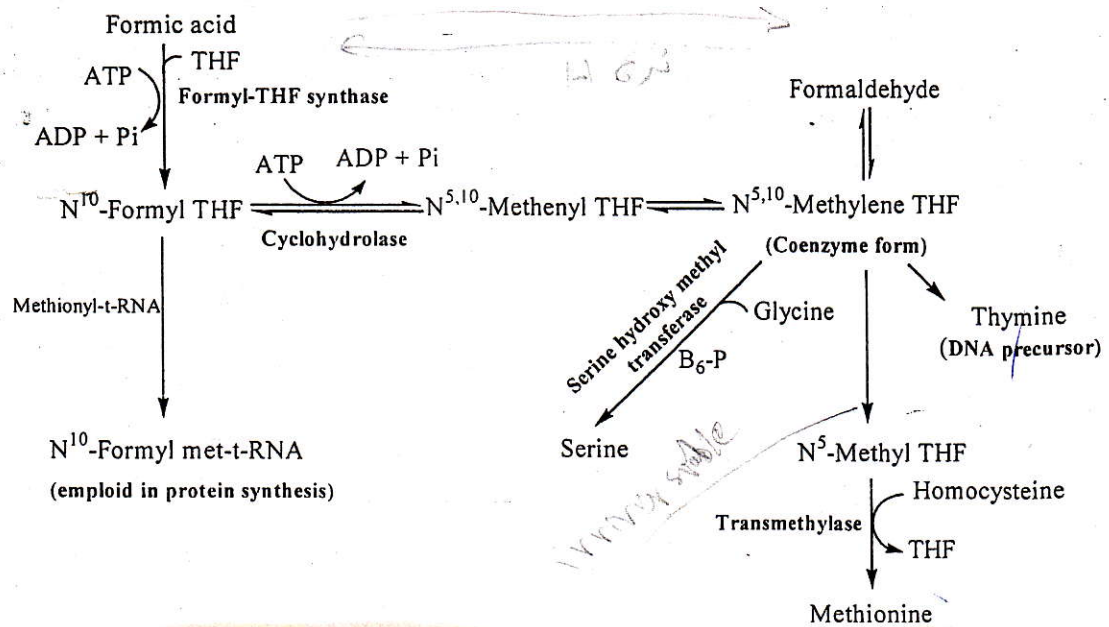


Figure: Structure of folic acid, dihydrofolic acid, and tetrahydrofolic acid

Folic acid is found in green leafy vegetables, liver, meat, whole grain cereals.

Folic acid plays a key role in one-carbon metabolism; it receives one-carbon fragments from donors such as serine, glycine, and histidine and transfers them to intermediates in the synthesis of amino acids, purines, and thymidine -the characteristic pyrimidine of DNA. The one-carbon unit may be: methyl ($-CH_3$), formyl (-CHO), formimino (-CH=NH), methylene ($-CH_2-$), methenyl (-CH=), hydroxymethyl ($-CH_2OH$).



Folic acid deficiency is characterized by growth failure and megaloblastic anemia.

Nutritional anemias:
Anemia is a condition in which the blood has a lower than normal concentration of hemoglobin, which results in a reduced ability to transport oxygen. Nutritional anemias—those caused by inadequate intake of one or more essential nutrients—can be classified according to the size of the red blood cells or mean corpuscular volume observed in the individual (Figure). [Note: These macrocytic anemias are commonly called megaloblastic because a deficiency of folic acid or vitamin B_{12} causes accumulation of large, immature red cell precursors, known as megaloblasts, in the bone marrow and the blood.]

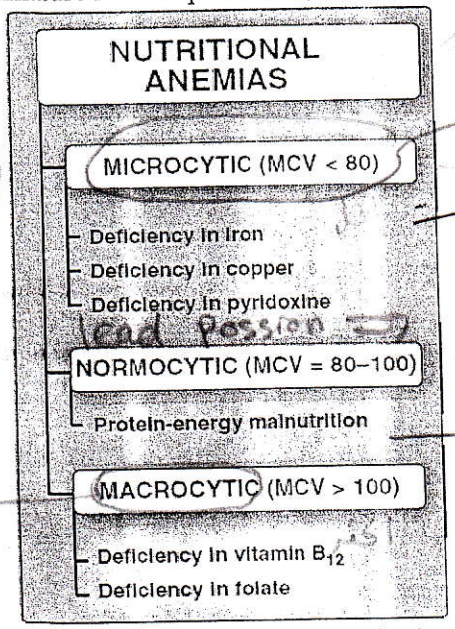


Figure: Classification of nutritional anemias by cell size. The normal mean corpuscular volume (MCV) for people older than age 18 is between 80 and 100 μm^3. [Note: Microcytic anemia is also seen with lead poisoning.]

Folate and anemia: Inadequate serum levels of folate can be caused by increased demand (for example, pregnancy and lactation), poor absorption caused by pathology of the small intestine, alcoholism, or treatment with drugs that are dihydrofolate reductase inhibitors, for example, methotrexate (Figure). A folate-free diet can cause a deficiency within a few weeks. A primary result of folic acid deficiency is megaloblastic anemia (Figure), caused by diminished synthesis of purines and TMP, which leads to an inability of cells (including red cell precursors) to make DNA and, therefore, they cannot divide. [Note: It is important to evaluate the cause of the megaloblastic anemia prior to instituting therapy, because vitamin B_{12} deficiency indirectly causes symptoms of this disorder]

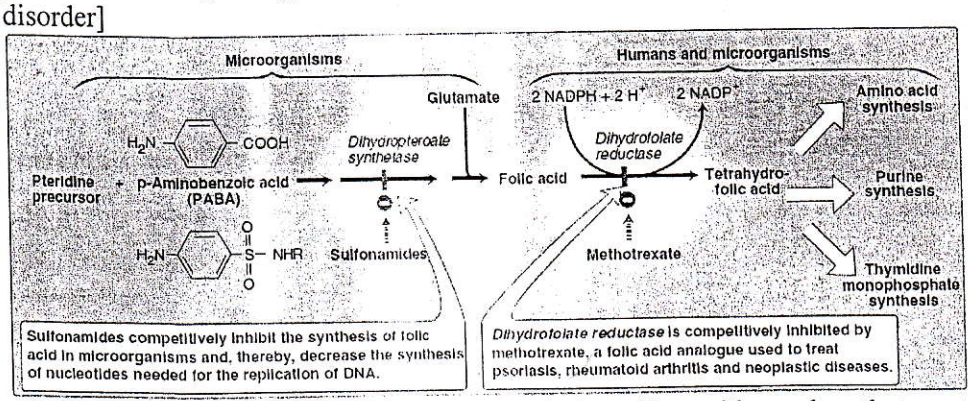


Figure: Inhibition of tetrahydrofolate synthesis by sulfonamides and methotrexate.

There is an association of high-dose supplementation with folic acid (>0.8 mg/day) and an increased risk of cancer. Thus, supplementation is not recommended for most middle-aged or older adults.

Cobalamin (Vitamin B_{12}):

Cobalamin contains a corrin ring system. Cobalt is held in the center of the corrin ring by four coordinate bonds. The remaining coordinate bonds of the cobalt are with the nitrogen of 5,6-dimethylbenzimidazole and with cyanide in commercial preparations of the vitamin in the form of **cyanocobalamin**. The coenzyme forms of cobalamin are **5′deoxyadenosylcobalamin**, in which cyanide is replaced with 5′ deoxyadenosine, and **methylcobalamin**, in which cyanide is replaced by a methyl group.

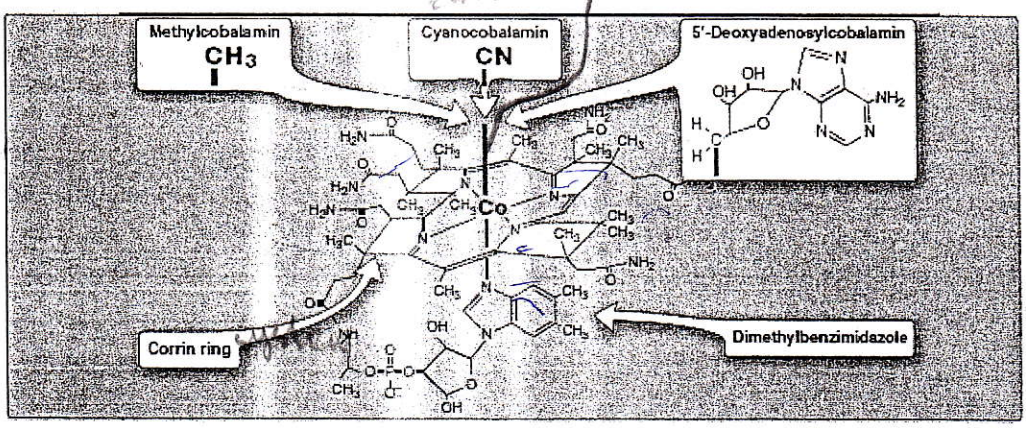
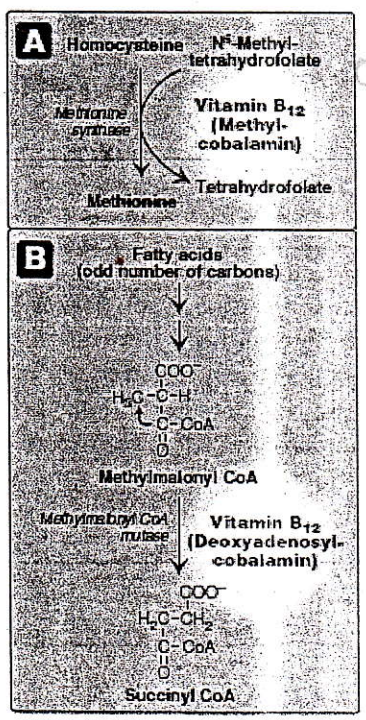


Figure: Structure of vitamin B_{12} (cyanocobalamin) and its coenzyme forms (methylcobalamin and 5'-deoxyadenosylcobalamin).



Vitamin B_{12} is required in humans for two essential enzymatic reactions: the remethylation of homocysteine to methionine and the isomerization of methylmalonyl coenzyme A (CoA) that is produced during the degradation of some amino acids (isoleucine, valine, threonine, and methionine), and fatty acids with odd numbers of carbon atoms (Figure). When the vitamin is deficient, unusual fatty acids accumulate and become incorporated into cell membranes, including those of the nervous system. This may account for some of the neurologic manifestations of vitamin B_{12} deficiency.

Distribution of cobalamin:

Vitamin B_{12} is synthesized only by microorganisms; it is not present in plants. Animals obtain the vitamin preformed from their natural bacterial flora or by eating foods derived from other animals. Cobalamin is present in appreciable amounts in liver, whole milk, eggs, oysters, fresh shrimp, pork, and chicken.

Figure: Reactions requiring coenzyme forms of vitamin B_{12}.

Pernicious anemia: Vitamin B_{12} deficiency is rarely a result of an absence of the vitamin in the diet. It is much more common to find deficiencies in patients who fail to absorb the vitamin from the intestine. Malabsorption of cobalamin in the elderly is most often due to reduced secretion of gastric acid and less efficient absorption of vitamin B_{12} from foods. A severe malabsorption of vitamin B_{12} leads to pernicious anemia. This disease is most commonly a result of an autoimmune destruction of the gastric parietal cells that are responsible for the synthesis of a glycoprotein called **intrinsic factor**.

Normally, vitamin B_{12} obtained from the diet binds to intrinsic factor in the stomach (Figure). The cobalamin– intrinsic factor complex travels through the gut and eventually binds to specific receptors on the surface of mucosal cells of the ileum. The bound cobalamin is transported into the mucosal cell and, subsequently, into the general circulation, where it is carried by B_{12}-binding proteins. Lack of intrinsic factor prevents the absorption of vitamin B_{12}, resulting in pernicious anemia.

Patients with cobalamin deficiency are usually anemic, but later in the development of the disease they show neuropsychiatric symptoms. The disease is treated by giving high-dose B_{12} orally, or intramuscular (IM) injection of cyanocobalamin. Deficiency of vitamin B_{12} can be measured by the level of methylmalonic acid in blood, which is elevated in individuals with low intake or decreased absorption of the vitamin.

Folic acid can partially reverse the hematologic abnormalities of B_{12} deficiency and, therefore, can mask a cobalamin deficiency. Thus, therapy of megaloblastic anemia is often initiated with folic acid and vitamin B_{12} until the cause of the anemia can be determined.

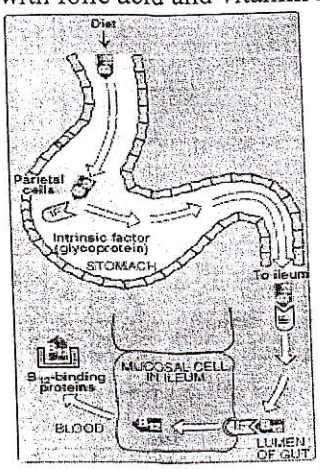


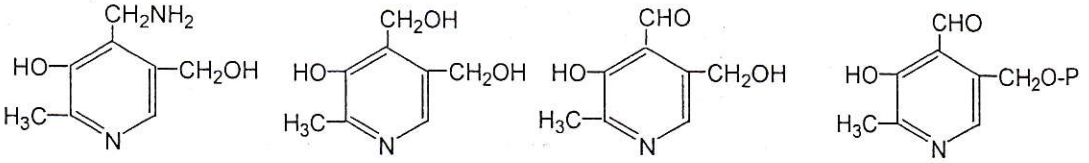
Figure: Absorption of vitamin B_{12}. IF =intrinsic factor.

Folate trap hypothesis:

The effects of cobalamin deficiency are most pronounced in rapidly dividing cells, such as the erythropoietic tissue of bone marrow and the mucosal cells of the intestine. Such tissues need both the N^5,N^{10}-methylene and N^{10}-formyl forms of tetrahydrofolate for the synthesis of nucleotides required for DNA replication. However, in vitamin B_{12} deficiency, the utilization of the N^5-methyl form of tetrahydrofolate in the B_{12}-dependent methylation of homocysteine to methionine is impaired. Because the methylated form cannot be converted directly to other forms of tetrahydrofolate, folate is trapped in the N^5-methyl form, which accumulates. The levels of the other forms decrease. Thus, cobalamin deficiency is hypothesized to lead to a deficiency of the tetrahydrofolate forms needed in purine and TMP synthesis, resulting in the symptoms of megaloblastic anemia.

Pyridoxal phosphate:

Vitamin B_6 is a collective term for pyridoxine, pyridoxal, and pyridoxamine, all derivatives of pyridine, differing only in the nature of the functional group attached to the ring. All three compounds can serve as precursors of the biologically active coenzyme, **pyridoxal phosphate** (pyridoxal-P).



Pyridoxamine **Pyridoxine** **Pyridoxal** **Pyridoxal phosphate**

Figure: Structure of vitamin B_6 and the coenzyme pyridoxal phosphate.

Pyridoxal-P is involved in a variety of reactions in the metabolism of amino acids including transamination, decarboxylation, deamination, and condensation.

Glutamate + Oxaloacetate ⟶ α-ketoglutarate + Aspartate

Histidine ⟶ Histamine + CO_2

Serine ⟶ Pyruvate + NH_3

Succinyl CoA + Glycine ⟶ δ-aminolevulinic acid

Good sources of the vitamin are wheat, corn, egg yolk, liver, meat.

Extreme deficiency of the vitamin causes convulsions due to the lowered activity of glutamic acid decarboxylase. As a result there occurs lowering of GABA in the brain which causes convulsions. Also, the vitamin deficiency causes a type of anemia called as a **hypochromic microcytic anemia** with high serum Fe^{++} level.

Isoniazid (isonicotinic acid hydrazide), a drug frequently used to treat tuberculosis, can induce a B_6 deficiency by forming an inactive derivative (hydrazone complex) with pyridoxal-P. Dietary deficiencies in pyridoxine are rare but have been observed in newborn infants fed formulas low in B_6, in women taking oral contraceptives, and in alcoholics.

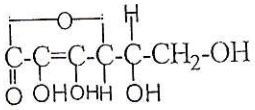
Toxicity of pyridoxine

Pyridoxine is the only water-soluble vitamin with significant toxicity. Neurologic symptoms (sensory neuropathy) occur at intakes above 200 mg/day.

Ascorbic acid (Vitamin C):

The active form of vitamin C is ascorbic acid (Figure). The main function of ascorbate is as a reducing agent in several different reactions. Vitamin C has a well documented role as a coenzyme in hydroxylation reactions, for example, hydroxylation of prolyl- and lysyl-residues of collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue, as well as for wound healing. Vitamin C also facilitates the absorption of dietary iron from the intestine.

Citrus fruits, potatoes (particularly their skins), tomatoes, and green vegetables are good sources for vitamin C.



Ascorbic acid

Figure: Structure of ascorbic acid.

A deficiency of vitamin C results in **scurvy**, a disease characterized by sore, spongy gums, loose teeth, fragile blood vessels, swollen joints, and anemia. Many of the deficiency symptoms can be explained by a deficiency in the hydroxylation of collagen, resulting in defective connective tissue.

Vitamin C is one of a group of nutrients, which includes vitamin E and β-carotene, which are known as **antioxidants**. Supplementation of the diet with these compounds decreases the incidence of chronic disease, such as coronary heart disease and certain cancers. That is because their biologic actions in the ability to inactivate toxic oxygen free radicals. Reactive oxygen radicals arise both as a byproduct of normal metabolism and by exposure to sunlight, ozone, tobacco smoke, and other environmental pollutants. Free radicals are known to damage lipid membranes, proteins, and cellular DNA; free radicals are thought to play a role in the development of heart and drug disease, cancer, and even aging. However, clinical trials involving supplementation with the isolated antioxidants have failed to determine any convincing beneficial effects.

Lipoic acid:

It was identified as a sulphur containing carboxylic acid called as 6,8-dithioctanoic acid (α-lipoic acid or thioctic acid). It contains eight carbon and two sulphur atoms. Lipoic acid exists in both oxidized and reduced forms due to the ability of the disulphide linkage to undergo reduction.

Lipoic acid (Reduced form) **Lipoic acid (Oxidized form)**

Figure: Structure of lipoic acid.

Lipoic acid is a coenzyme of the multienzyme complexes **pyruvate dehydrogenase** and **α-ketoglutarate dehydrogenase**. It is required along with other coenzymes in oxidative decarboxylation of pyruvic acid to acetyl CoA, and of α-ketoglutarate to succinyl CoA.

The chemical aspect of the coenzymatic action of α-lipoic acid is to mediate the transfer of electrons and activated acyl groups resulting from the decarboxylation and oxidation of α-keto acids (i.e. pyruvate, α-ketoglutarate) within the complexes.

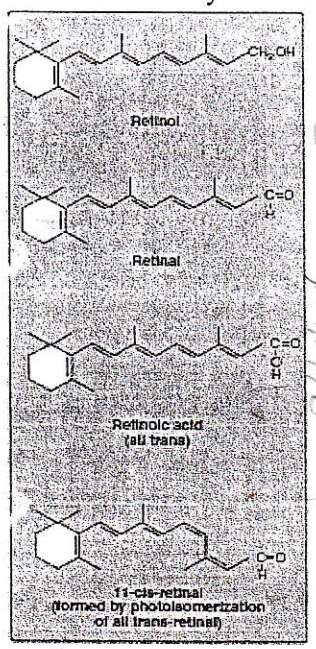
Liver and yeast are rich sources of lipoic acid.

Vitamin A:

The retinoids, a family of molecules that are related to retinol (vitamin A), are essential for vision, reproduction, growth, and maintenance of epithelial tissues. Retinoic acid, derived from oxidation of dietary retinol, mediates most of the actions of the retinoids, except for vision, which depends on retinal, the aldehyde derivative of retinol.

Structure of vitamin A:

Vitamin A is often used as a collective term for several related biologically active molecules (Figure). The term retinoids includes both natural and synthetic forms of vitamin A that may or may not show vitamin A activity.



1) **Retinol:** A primary alcohol containing a β-ionone ring with an unsaturated side chain, retinol is found in animal tissues as a retinyl ester with long-chain fatty acids.

2) **Retinal:** This is the aldehyde derived from the oxidation of retinol. Retinal and retinol can readily be interconverted.

3) **Retinoic acid:** This is the acid derived from the oxidation of retinal. Retinoic acid cannot be reduced in the body, and, therefore, cannot give rise to either retinal or retinol.

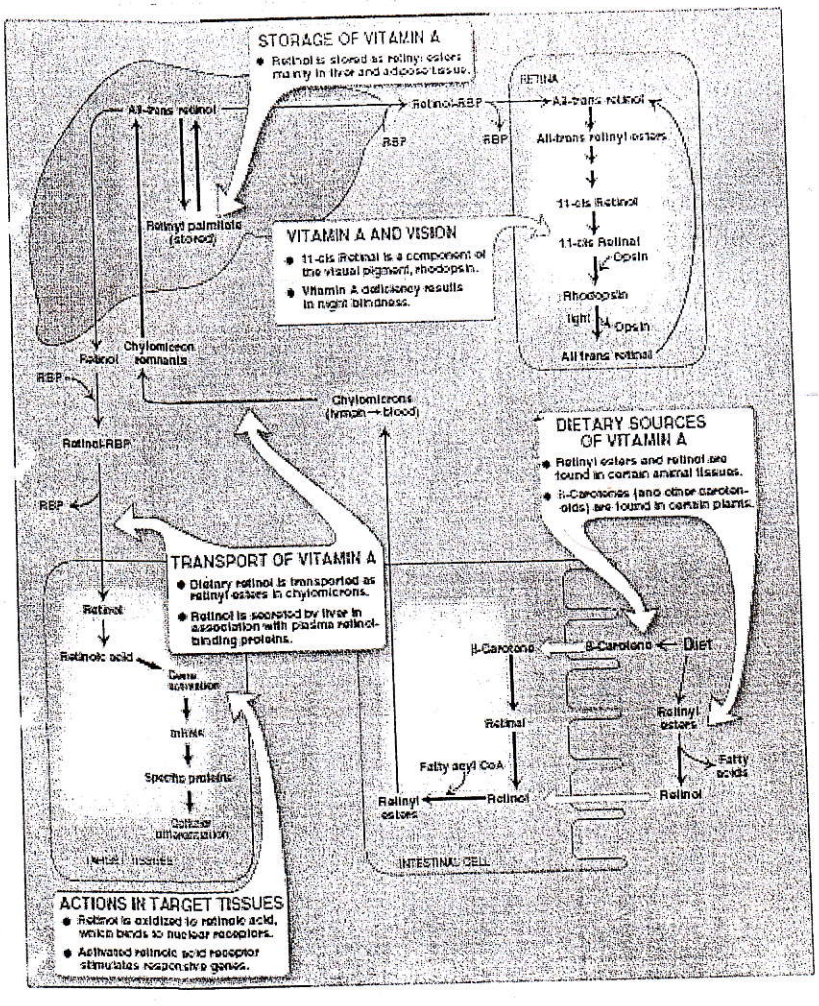
4) **β-Carotene:** Plant foods contain β-carotene, which can be oxidatively cleaved in the intestine to yield two molecules of retinal. In humans, the conversion is inefficient, and the vitamin A activity of β-carotene is only about one twelfth that of retinol.

Figure: Structure of the retinoids.

Absorption and transport of vitamin A:

1) **Transport to the liver:** Retinol esters present in the diet are hydrolyżed in the intestinal mucosa, releasing retinol and free fatty acids (Figure). Retinol derived from esters and from the cleavage and reduction of carotenes is re-esterified to long-chain fatty acids in the intestinal mucosa and secreted as a component of chylomicrons into the lymphatic system . Retinol esters contained in chylomicron remnants are taken up by, and stored in, the liver.

2) **Release from the liver:** When needed, retinol is released from the liver and transported to extrahepatic tissues by the plasma retinol-binding protein (RBP). The retinol–RBP complex attaches to specific receptors on the surface of the cells of peripheral tissues, permitting retinol to enter. Many tissues contain a cellular retinol-binding protein that carries retinol to sites in the nucleus where the vitamin acts in a manner analogous to that of steroid hormones.



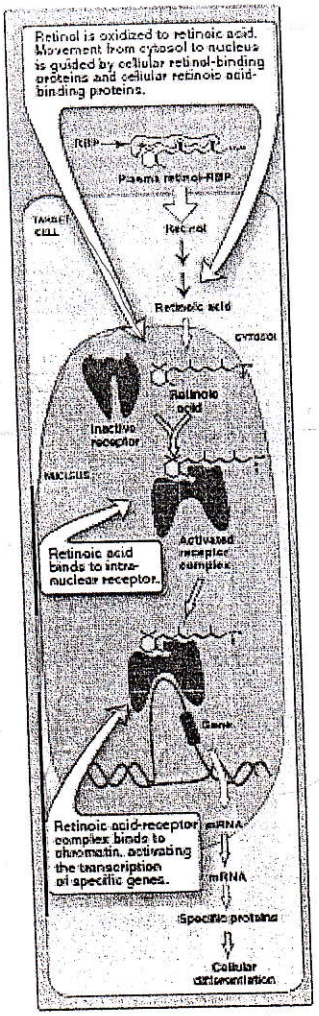


Figure: Absorption, transport, and storage of vitamin A and its derivatives.

RBP = retinol-binding protein.

Figure: Action of retinoids Note: Retinoic acid-receptor complex is a dimer, but is shown as monomer for simplicity.[RBP = retinol-binding protein.]

Mechanism of action of vitamin A:

Retinoic acid binds with high affinity to specific receptor proteins present in the nucleus of target tissues, such as epithelial cells (Figure). The activated retinoic acid–receptor complex interacts with nuclear chromatin to stimulate retinoid-specific RNA synthesis, resulting in the production of specific proteins that mediate several physiologic functions. For example, retinoids control the expression of the keratin gene in most epithelial tissues of the body. The specific retinoic acid–receptor proteins are part of the super family of transcriptional regulators that includes the steroid and thyroid hormones and 1,25-dihydroxycholecalciferol, all of which function in a similar way.

Functions of vitamin A:

1) Visual cycle: Vitamin A is a component of the visual pigments of rod and cone cells. Rhodopsin, the visual pigment of the rod cells in the retina, consists of 11-cis retinal specifically bound to the protein opsin. When rhodopsin is exposed to light, a series of photochemical isomerizations occurs, which results in the bleaching of the visual pigment and release of all trans retinal and opsin. This process triggers a nerve impulse that is transmitted by the optic nerve to the brain. Regeneration of rhodopsin requires isomerization of all trans retinal back to 11-cis retinal. Trans retinal, after being released from rhodopsin, is isomerized to 11-cis retinal, which spontaneously combines with opsin to form rhodopsin, thus completing the cycle. Similar reactions are responsible for color vision in the cone cells.

2) Growth: Vitamin A deficiency results in a decreased growth rate in children. Bone development is also slowed.

3) Reproduction: Retinol and retinal are essential for normal reproduction, supporting spermatogenesis in the male and preventing fetal resorption in the female. Retinoic acid is inactive in maintaining reproduction and in the visual cycle, but promotes growth and differentiation of epithelial cells; thus, animals given vitamin A only as retinoic acid from birth are blind and sterile.

4) Maintenance of epithelial cells: Vitamin A is essential for normal differentiation of epithelial tissues and mucus secretion.

Distribution of vitamin A:
Liver, kidney, cream, butter, and egg yolk are good sources of preformed vitamin A. Yellow and dark green vegetables and fruits are good dietary sources of the carotenes, which serve as precursors of vitamin A.

Requirement for vitamin A:
The RDA for adults is 900 retinol activity equivalents (RAE) for males and 800 RAE for females. In comparison, 1 RAE = 1 mg of retinol, 12 mg of β-carotene, or 24 mg of other carotenoids.

Clinical indications:

1) Dietary deficiency: Vitamin A, administered as retinol or retinyl esters, is used to treat patients who are deficient in the vitamin (Figure). Night blindness is one of the earliest signs of vitamin A deficiency. The visual threshold is increased, making it difficult to see in dim light. Prolonged deficiency leads to an irreversible loss in the number of visual cells. Severe vitamin A deficiency leads to xerophthalmia, a pathologic dryness of the conjunctiva and cornea. If untreated, xerophthalmia results in corneal ulceration and, ultimately, in blindness because of the formation of opaque scar tissue. The condition is most frequently seen in children in developing tropical countries. Over 500,000 children worldwide are blinded each year by xerophthalmia caused by insufficient vitamin A in the diet.

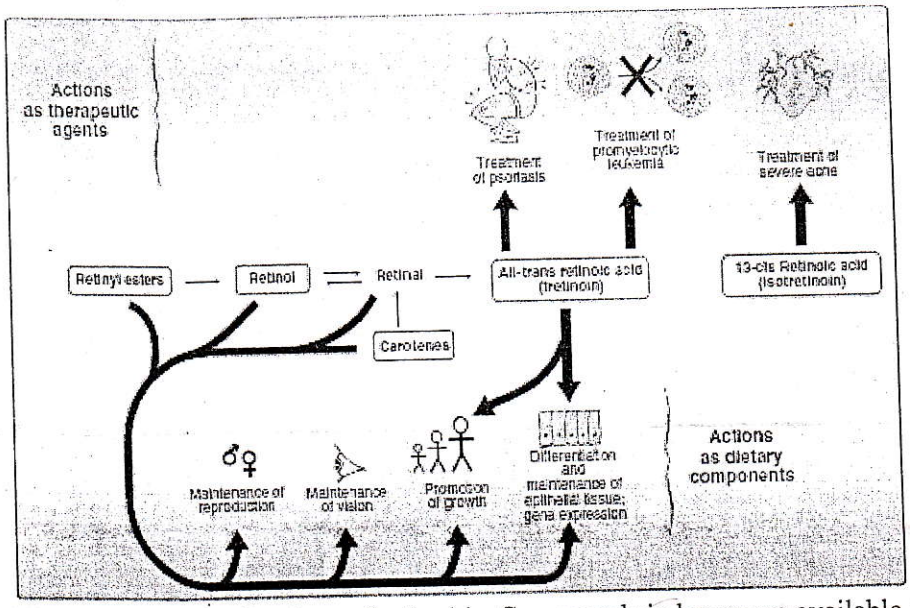


Figure: Summary of actions of retinoids. Compounds in boxes are available as dietary components or as pharmacologic agents.

2) **Acne and psoriasis:** Dermatologic problems such as acne and psoriasis are effectively treated with retinoic acid or its derivatives (Figure). Mild cases of acne, Darier disease (keratosis follicularis), and skin aging are treated with topical application of tretinoin (all-trans retinoic acid), as well as benzoyl peroxide and antibiotics. [Note: Tretinoin is too toxic for systemic administration and is confined to topical application.] In patients with severe recalcitrant cystic acne unresponsive to conventional therapies, the drug of choice is isotretinoin (13-cis retinoic acid) administered orally.

Toxicity of retinoids:

1) **Vitamin A:** Excessive intake of vitamin A produces a toxic syndrome called hypervitaminosis A. Amounts exceeding 7.5 mg/day of retinol should be avoided. Early signs of chronic hypervitaminosis A are reflected in the skin, which becomes dry and pruritic, the liver, which becomes enlarged and can become cirrhotic, and in the nervous system, where a rise in intracranial pressure may mimic the symptoms of a brain tumor. Pregnant women particularly should not ingest excessive quantities of vitamin A because of its potential for causing congenital malformations in the developing fetus.

2) **Isotretinoin:** The drug is teratogenic and absolutely contraindicated in women with childbearing potential unless they have severe, disfiguring cystic acne that is unresponsive to standard therapies. Pregnancy must be excluded before initiation of treatment, and adequate birth control must be used. Prolonged treatment with isotretinoin leads to hyperlipidemia and an increase in the LDL/HDL ratio, providing some concern for an increased risk of cardiovascular disease.

Vitamin D:

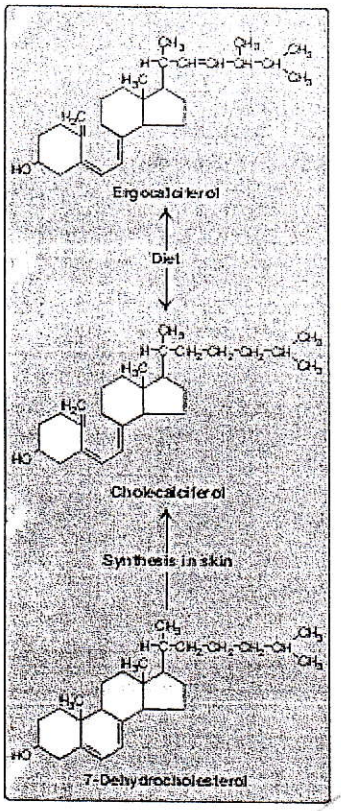


Figure: Sources of Vitamin D

A. Distribution and requirement of vitamin D

Vitamin D occurs naturally in fatty fish, liver, and egg yolk. Milk, unless it is artificially fortified, is not a good source of the vitamin. AI for vitamin D is 200 IU to age 50, and 400-600 IU after age 50.

B. Distribution of vitamin D

1. Diet: Ergocalciferol (vitamin D_2), found in plants, and cholecalciferol (vitamin D_3), found in animal tissues, are sources of preformed vitamin D activity (Figure).

2. Endogenous vitamin precursor: 7-Dehydrocholesterol, an intermediate in cholesterol synthesis, is converted to cholecalciferol in the dermis and epidermis of humans exposed to sunlight

C. Metabolism of vitamin D

1. Formation of 1,25-diOH-D_3: Vitamins D_2 and D_3 are not biologically active, but are converted in vivo to the active form of the D vitamin by two sequential hydroxylation reactions (Figure). The first hydroxylation occurs at the 25-position, and is catalyzed by a specific hydroxylase in the liver. The product of the reaction,25-hydroxy cholecalciferol (25-OH-D_3, calcidol), is the predominant form of vitamin D in the plasma and the major storage form of the vitamin. 25-OH-D_3 is further hydroxylated at the 1 position by 25-hydroxy cholecalciferol 1-hydroxylase found primarily in the kidney, resulting in the formation of 1,25-diOH-D_3 (calcitriol).

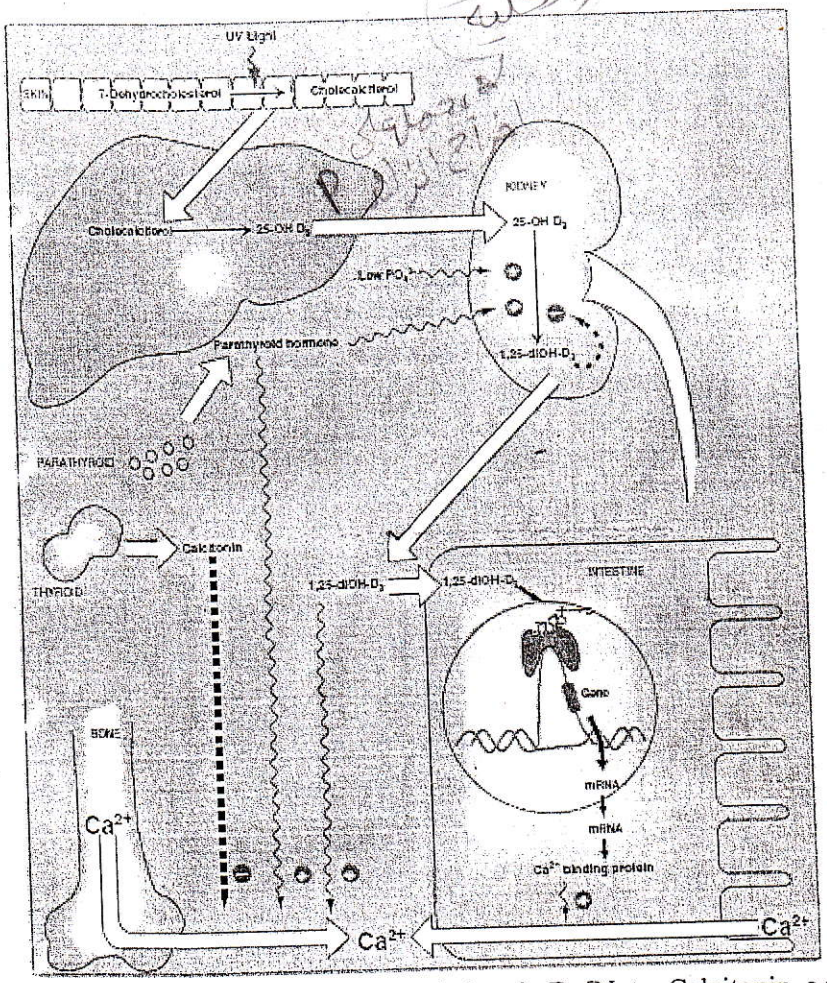


Figure : Metabolism and actions of vitamin D. [Note: Calcitonin, a thyroid hormone, decreases blood calcium by inhibiting mobilization from bone and reabsorption by the kidney.]

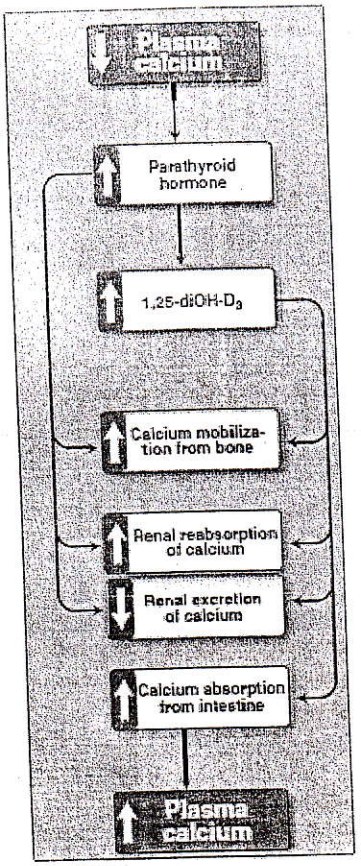


Figure: Response to low serum calcium

2. Regulation of 25-hydroxycholecalciferol 1-hydroxylase: 1,25- diOH-D_3 is the most potent vitamin D metabolite. Its formation is tightly regulated by the level of plasma phosphate and calcium ions (Figure). 25-Hydroxycholecalciferol 1-hydroxylase activity is increased directly by low plasma phosphate or indirectly by low plasma calcium, which triggers the release of parathyroid hormone (PTH). Hypocalcemia caused by insufficient dietary calcium thus results in elevated levels of plasma 1,25-diOH-D_3. 1-Hydroxylase activity is also decreased by excess 1,25-diOH-D_3, the product of the reaction.

D. Function of vitamin D

The overall function of 1,25-diOH-D_3 is to maintain adequate plasma levels of calcium. It performs this function by: **1)** increasing uptake of calcium by the intestine, **2)** minimizing loss of calcium by the kidney, and **3)** stimulating resorption of bone when necessary.

1,25-diOH-D_3 stimulates intestinal absorption of calcium and phosphate. 1,25-diOH-D_3 enters the intestinal cell and binds to a cytosolic receptor. The 1,25-diOH-D_3–receptor complex then moves to the nucleus where it selectively interacts with the cellular DNA. As a result, calcium uptake is enhanced by an increased synthesis of a specific calcium-binding protein. Thus, the mechanism of action of 1,25-diOH-D_3 is typical of steroid hormones.

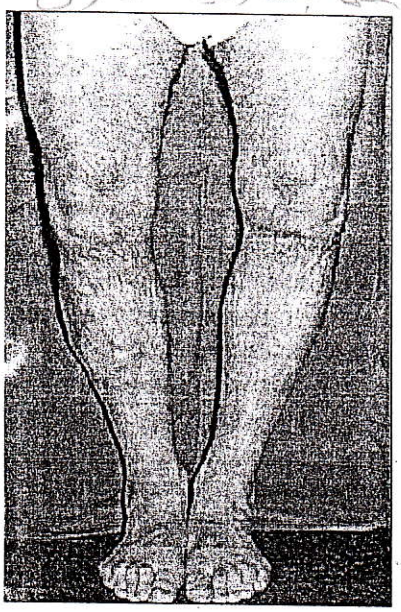


Figure: Bowed legs of middle-aged man with osteomalacia, a nutritional vitamin D deficiency that results in malformation of the skeleton.

E. Clinical indications

1. Nutritional rickets: Vitamin D deficiency causes a net de - mineralization of bone, resulting in rickets in children and osteomalacia in adults (Figure). Rickets is characterized by the continued formation of the collagen matrix of bone, but incomplete mineralization, resulting in soft, pliable bones. In osteomalacia, demineralization of pre-existing bones increases their susceptibility to fracture. Insufficient exposure to daylight and/or deficiencies in vitamin D consumption occur predominantly in infants and the elderly. [Note: The recommended intake of 200 IU/day (which corresponds to 5 µg of cholecalciferol) may be insufficient, because higher doses of 800 IU/day have been shown to reduce the incidence of osteoporotic fractures.] Sufficient levels of serum 25-hydroxycholecalciferol (>75 nmol/L) have been linked to fall prevention in older people as well as to increased muscle strength and bone mass.

2. Renal osteodystrophy: Chronic renal failure results in decreased ability to form the active form of vitamin D. Supplementation with calcitriol is an effective therapy. [Note: Vitamin D supplementation is accompanied by phosphate reduction therapy to prevent hyperphosphatemia (due to renal failure) and precipitation of calcium phosphate crystals.]

3. Hypoparathyroidism: Lack of parathyroid hormone causes hypocalcemia and hyperphosphatemia. These patients may be treated with calcitriol and calcium supplementation.

F. Toxicity of vitamin D

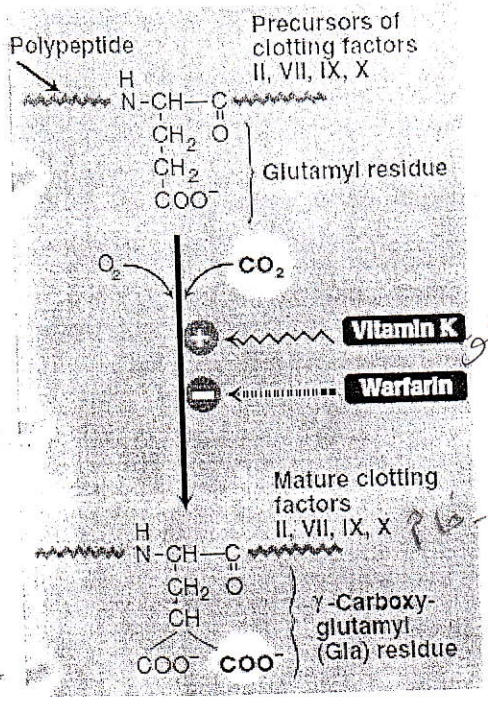
Like all fat-soluble vitamins, vitamin D can be stored in the body and is only slowly metabolized. High doses (100,000 IU for weeks or months) can cause loss of appetite, nausea, thirst, and stupor. Enhanced calcium absorption and bone resorption results in hypercalcemia, which can lead to deposition of calcium in many organs, particularly the arteries and kidneys. The UL is 2000 IU/day.

VITAMIN K

Distribution and requirement of vitamin K

Vitamin K is found in cabbage, kale, spinach, egg yolk, and liver. There is also extensive synthesis of the vitamin by the bacteria in the gut. AI for vitamin K is 120 µg/day for adult males and 90 µg for adult females.

The principal role of vitamin K is in the posttranslational modification of various blood clotting factors, in which it serves as a coenzyme in the carboxylation of certain glutamic acid residues present in these proteins. Vitamin K exists in several forms, for example, in plants as phylloquinone (or vitamin K_1), and in intestinal bacterial flora as menaquinone (or vitamin K_2). A synthetic form of vitamin K, menadione, is available.



Function of vitamin K

1. Formation of γ-carboxyglutamate (Gla): Vitamin K is required in the hepatic synthesis of prothrombin and blood clotting factors II, VII, IX, and X. These proteins are synthesized as inactive precursor molecules. Formation of the clotting factors requires the vitamin K–dependent carboxylation of glutamic acid residues to Gla residues (Figure). This forms a mature clotting factor that contains Gla and is capable of subsequent activation. The reaction requires O_2, CO_2, and the hydroquinone form of vitamin K. The formation of Gla is sensitive to inhibition by dicumarol, an anticoagulant occurring naturally in spoiled sweet clover, and by warfarin, a synthetic analog of vitamin K.

Figure: Carboxylation of glutamate to form γ-carboxyglutamate (Gla).

2. Interaction of prothrombin with platelets: The Gla residues of prothrombin are good chelators of positively charged calcium ions, because of the two adjacent, negatively charged carboxylate groups. The prothrombin–calcium complex is then able to bind to phospholipids essential for blood clotting on the surface of platelets. Attachment to the platelet increases the rate at which the proteolytic conversion of prothrombin to thrombin can occur

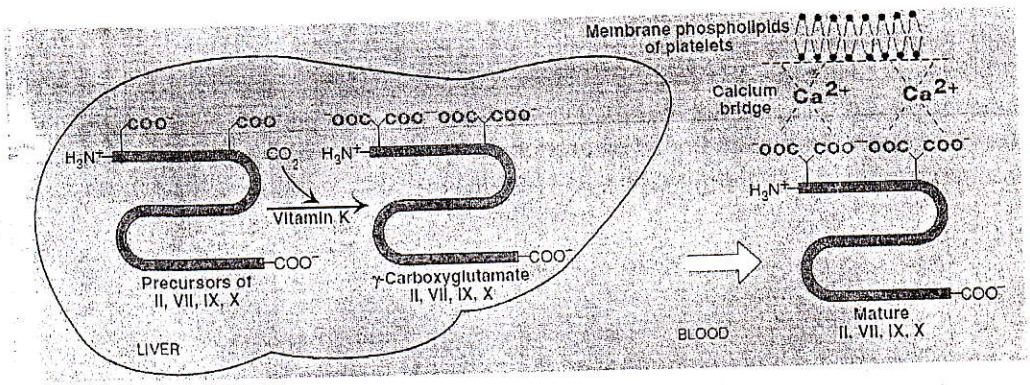


Figure: Role of vitamin K in blood coagulation.

3. Role of Gla residues in other proteins: Gla is also present in other proteins (for example, osteocalcin of bone, and in proteins such as protein C involved in limiting the formation of blood clots).

Clinical indications

1. Deficiency of vitamin K: A true vitamin K deficiency is unusual because adequate amounts are generally produced by intestinal bacteria or obtained from the diet. If the bacterial population in the gut is decreased, for example, by antibiotics, the amount of endogenously formed vitamin is depressed, and this can lead to hypoprothrombinemia in the marginally malnourished individual, for example, a debilitated geriatric patient. This condition may require supplementation with vitamin K to correct the bleeding tendency. In addition, certain second-generation cephalosporins, for example, cefoperazone, cefamandole, and moxalactam cause hypoprothrombinemia, apparently by a warfarin-like mechanism. Consequently, their use in treatment is usually supplemented with vitamin K.

2. Deficiency of vitamin K in the newborn: Newborns have sterile intestines and so initially lack the bacteria that synthesize vitamin K. Because human milk provides only about one fifth of the daily requirement for vitamin K, it is recommended that all newborns receive a single intramuscular dose of vitamin K as prophylaxis against hemorrhagic disease.

Toxicity of vitamin K

Prolonged administration of large doses of synthetic vitamin K (menadione) can produce hemolytic anemia and jaundice in the infant, due to toxic effects on the membrane of red blood cells; therefore, it is no longer used to treat vitamin K deficiency.

VITAMIN E

The E vitamins consist of eight naturally occurring tocopherols, of which α-tocopherol is the most active (Figure). The primary function of vitamin E is as an antioxidant in prevention of the nonenzymic oxidation of cell components, for example, polyunsaturated fatty acids, by molecular oxygen and free radicals.

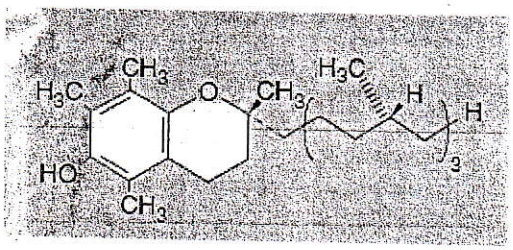


Figure: Structure of vitamin E.

Distribution and requirements of vitamin E

Vegetable oils are rich sources of vitamin E, whereas liver and eggs contain moderate amounts. The RDA for α -tocopherol is 15 mg for adults. The vitamin E requirement increases as the intake of polyunsaturated fatty acid increases.

Deficiency of vitamin E

Vitamin E deficiency is almost entirely restricted to premature infants. When observed in adults, it is usually associated with defective lipid absorption or transport. The signs of human vitamin E deficiency include sensitivity of erythrocytes to peroxide, and the appearance of abnormal cellular membranes.

Clinical indications

Vitamin E is not recommended for the prevention of chronic disease, such as coronary heart disease or cancer. Clinical trials using vitamin E supplementation have been uniformly disappointing. For example, subjects in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study trial who received high doses of vitamin E not only lacked cardiovascular benefit but also had an increased incidence of stroke.

Populations consuming diets high in fruits and vegetables show decreased incidence of some chronic diseases. However, clinical trials have failed to show a definitive benefit from supplements of vitamins A, C, or E; multivitamins with folic acid; or antioxidant combinations for the prevention of cancer or cardiovascular disease.

Toxicity of vitamin E

Vitamin E is the least toxic of the fat-soluble vitamins, and no toxicity has been observed at doses of 300 mg/day.

VITAMIN	OTHER NAMES	ACTIVE FORM	FUNCTION
Folic acid	—	Tetrahydrofolic acid	Transfer one-carbon units; Synthesis of methionine, purines, and thymidine monophosphate
Vitamin B_{12}	Cobalamin	Methylcobalamin Deoxyadenosyl cobalamin	Coenzyme for reactions: Homocysteine → methionine Methylmalonyl CoA → succinyl CoA
Vitamin C	Ascorbic acid	Ascorbic acid	Antioxidant Coenzyme for hydroxylation reactions, for example: In procollagen: Proline → hydroxyproline Lysine → hydroxylysine
Vitamin B_6	Pridoxine Pyridoxamine Pyridoxal	Pyridoxal phosphate	Coenzyme for enzymes, particularly in amino acid metabolism
Vitamin B_1	Thiamine	Thiamine pyrophosphate	Coenzyme of enzymes catalyzing: Pyruvate → acetyl CoA α-Ketoglutarate → Succinyl CoA Ribose 5-P + xylulose 5-P → Sedoheptulose 7-P + Glyceraldehyde 3-P Branched-chain amino acid oxidation
Niacin	Nicotinic acid Nicotinamide	NAD^+, $NADP^+$	Electron transfer
Vitamin B_2	Riboflavin	FMN, FAD	Electron transfer
Biotin	—	Enzyme-bound biotin	Carboxylation reactions
Pantothenic acid	—	Coenzyme A	Acyl carrier WATER-SOLUBLE
Vitamin A	Retinol Retinal Retinoic acid β-Carotene	Retinol Retinal Retinoic acid	FAT-SOLUBLE Maintenance of reproduction Vision Promotion of growth Differentiation and maintenance of epithelial tissues Gene expression
Vitamin D	Cholecalciferol Ergocalciferol	1,25-Dihydroxycholecalciferol	Calcium uptake
Vitamin K	Menadione Menaquinone Phylloquinone	Menadione Menaquinone Phylloquinon	γ-Carboxylation of glutamate residue in clotting and other proteins
Vitamin E	α-Tocopherol	Any of several tocopherol derivatives	Antioxidant

DEFICIENCY	SIGNS AND SYMPTOMS	TOXICITY	NOTES
Megaloblastic anemia Neural tube defects	Anemia Birth defects	None	Administration of high levels of folate can mask vitamin B_{12} deficiency
Pernicious anemia Dementia Spinal degeneration	Megaloblastic anemia Neuropsychiatric symptoms	None	Pernicious anemia is treated with IM or high-dose oral vitamin B_{12}
Scurvy	Sore, spongy gums Loose teeth Poor wound healing	None	Benefits of supplementation not established in controlled trials
Rare	Glossitis Neuropathy	Yes	Deficiency can be induced by isoniazid Sensory neuropathy occurs at high doses
Beriberi Wernicke-Korsakoff syndrome (most common in alcoholics)	Tachycardia, vomiting, convulsions Apathy, loss of memory, eye movements	None	—
Pellagra	Dermatitis Diarrhea Dementia	None	High doses of niacin used to treat hyperlipidemia
Rare	Dermatitis Angular stomatitis	None	—
Rare	—	None	Consumption of large amounts of raw egg whites (which contains a protein, avidin, that binds biotin) can induce a biotin deficiency
Rare	—	None	—
			WATER-SOLUBLE
			FAT-SOLUBLE
Infertility Night blindness Retardation of growth Xerophthalmia	Increased visual threshold Dryness of cornea	Yes	β-Carotene not acutely toxic, but supplementation is not recommended Excess vitamin A can increase incidence of fractures
Rickets (in children) Osteomalacia (in adults)	Soft, pliable bones	Yes	Vitamin D is not a true vitamin because it can be synthesized in skin. Application of sunscreen lotions or presence of dark skin color decreases this synthesis.
Newborn Rare in adults	Bleeding	Rare	Vitamin K produced by intestinal bacteria. Vitamin K deficiency common in newborns Intramuscular treatment with vitamin K is recommended at birth
Rare	Red blood cell fragility leads to hemolytic anemia	None	Benefits of supplementation not established in controlled trials

METABOLISM OF CARBOHYDRATES

DIGESTION & ABSORPTION OF CARBOHYDRATE

Digestion in mouth:

Digestion of carbohydrate starts at the mouth. In mouth, saliva contains salivary *α-amylase* (*Ptyalin*) which requires Cl^- ions for activation and optimum pH 6.7. The enzyme randomly hydrolyzes α-1→4 glycosidic linkages in polysaccharide molecule (starch and glycogen).

Digestion in stomach:

No carbohydrate splitting enzymes available in gastric juice.

Digestion in duodenum:

Food reaches the duodenum from stomach where it meets the pancreatic juice, which contains an enzyme called as *pancreatic amylase* (*Amylopsin*) similar to salivary amylase. The enzyme hydrolyzes α-1→4 glycosidic linkages in polysaccharides and requires Cl^- ions for activation and optimum pH 7.1

Digestion in Small Intestine:

Action of Intestinal Juice:

Intestinal amylase:

This enzyme hydrolyzes α-1→ 4 glycosidic linkages in polysaccharide and oligosaccharide molecules liberating free glucose molecules.

Lactase:

$$\text{Lactose} \xrightarrow{\textit{Lactase}} \text{Glucose} + \text{Galactose}$$

Isomaltase:

It catalyzes the hydrolysis of α-1→ 6 glycosidic linkages, thus splitting α-limit dextrin at the branching points and producing maltose and glucose.

Maltase:

$$\text{Maltose} \xrightarrow{\textit{Maltase}} \text{Glucose} + \text{Glucose}$$

Sucrase:

$$\text{Sucrose} \xrightarrow{\textit{Sucrase}} \text{Glucose} + \text{Fructose}$$

Absorption of Carbohydrates:

No carbohydrates higher than monosaccharides can be absorbed directly into the blood stream in normal health.

Insulin is not required for the uptake of glucose by intestinal cells. However, different sugars have different mechanisms of absorption. For example, galactose and glucose are transported into the mucosal cells by an active transport that requires a carrier protein, ATP, and Na^+ ion (bind to

the carrier protein and changes the conformation of the protein, enabling the binding to take place and thus the absorption of glucose to occur).

Fructose and mannose are absorbed by facilitated transport which requires a protein carrier and no need for ATP.

After absorption all monosaccharides reaches the liver via portal vein. In liver fructose and also galactose are converted to glucose. Then, from liver glucose go to circulation where taken up by extrahepatic cells with the help of insulin.

Any defect in a specific disaccharidase activity of the intestinal mucosa causes the passage of undigested carbohydrate into the large intestine. As a consequence of the presence of this osmotically active material, water is drawn from the mucosa into the large intestine, causing osmotic diarrhea. This is reinforced by the bacterial fermentation of the remaining carbohydrate of two- and three-carbon compounds (which are also osmotically active) plus large volumes of CO_2 and H_2 gas, causing flatulence.

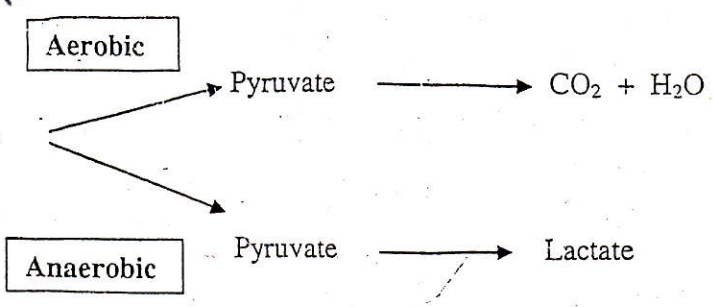
CARBOHYDRATE METABOLISM

Glycolysis:

Definition: The degradation (oxidation) of glucose to pyruvate.

Glycolysis is a cytosolic process occurs in all tissues.

Glycolyis is a unique process, since it can utilize O_2 if available (aerobic), and it can function in absence of O_2 (anaerobic).



Glucose is freely permeable to liver cells. RBCs entirely depends on glycolysis for its energy. Glucose is freely permeable to RBCs like liver cells. In intestinal mucosa and kidney tubules glucose is taken up by active transport. In other tissues like skeletal muscles, cardiac muscles, diaphragm, adipose tissue, etc., insulin facilitates the uptake of glucose.

Inside the cell, glucose is then phosphorylated to form glucose-6-P. (1 ATP molecule is consumed). The reaction is catalyzed by the specific enzyme ***glucokinase*** in liver cells, and by non-specific ***hexokinase*** in liver and extrahepatic tissues.

Hexokinase has a high affinity (low K_m) for its substrate (glucose). This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low. *Hexokinase* is not specific; it can phosphorylate any of the hexoses. *Hexokinase*, however, has a low V_{max} for glucose and therefore cannot phosphorylate large quantities of glucose. The enzyme is allosterically inhibited by glucose-6-P.

Glucokinase function to remove glucose from the blood following a meal. It functions only when the intracellular concentration of glucose in the hepatocyte is elevated, such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein. It has a high K_m for glucose and operates optimally at blood glucose concentrations above 100 mg/dl. *Glucokinase* has a high V_{max}, allowing the liver to effectively remove this flood of glucose from the portal blood. *Glucokinase* is specific for glucose and not inhibited by glucose-6-P.

Glucose-6-P is converted to fructose-6-P by the enzyme *phosphoglucose isomerase*.

Fructose-6-P is phosphorylatd with ***phosphofructokinase (PFK)*** to fructose-1,6-bisphosphate (another ATP molecule is consumed). PFK is the key enzyme in glycolysis which regulates breakdown of glucose.

Aldolase A cleaves fructose-1,6-bisphosphate to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-P. DHAP must be isomerized to glyceraldehyde-3-P by *phosphotriose isomerase* for further metabolism in the glycolytic sequence.

Glycolysis proceed by the oxidation of glyceraldehyde-3-P to 1,3-Diphosphoglycerate by *glyceraldehyde-3-P dehydrogenase* (NAD^+-dependent). 2 molecules of NADH are generated at this stage per molecule of glucose.

The high-energy phosphate group of 1,3-diphosphoglycerate is used to synthesis ATP from ADP in a reaction catalyzed by *phosphoglycerate kinase* (2 ATP molecules are obtained). This reaction is called **substrate-level phosphorylation** because ATP is formed directly from the oxidation of the substrate instead of resulting from oxidative phosphorylation via the electron transport chain.

3-Phosphoglycerate is converted to 2-phosphoglycerate by the enzyme *phosphoglycerate mutase*.

Phosphoenolpyruvate (PEP) is formed as a result of the dehydration of 2-phosphoglycerate by *enolase*.

The conversion of PEP to pyruvate is catalyzed by ***pyruvate kinase***. 2 ATP molecules / glucose molecule are formed (a second substrate-level phosphorylation).

In absence of oxygen (anaerobic glycolysis), pyruvate is reduced by NADH to lactate by the action of ***lactate dehydrogenase***. This allows glycolysis to proceed in the absence of O_2 by regeneration of sufficient NAD^+ for another cycle of the reaction catalyzed by *glyceraldehyde-3-P dehydrogenase.*

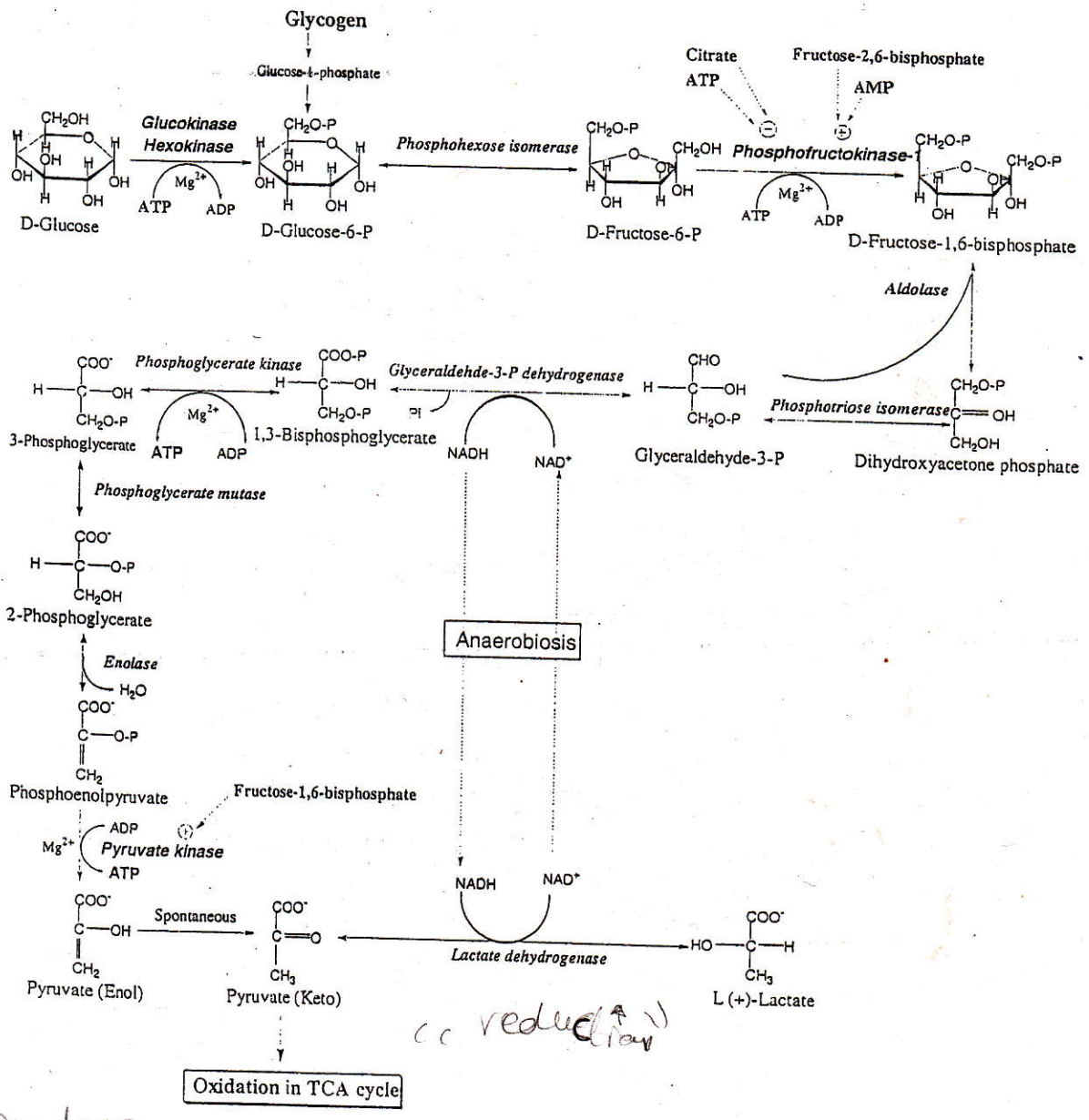


Figure: Glycolysis pathway.

Glycolysis in RBCs, even under aerobic conditions, always terminates in lactate due to the absence of mitochondria.

In presence of oxygen (aerobic glycolysis), pyruvate formed in cytosol enters mitochondria and oxidatively decarboxylated to acetyl-CoA by ***pyruvate dehydrogenase complex***. Acetyl-CoA now is ready to enter citric acid cycle (TCA cycle).

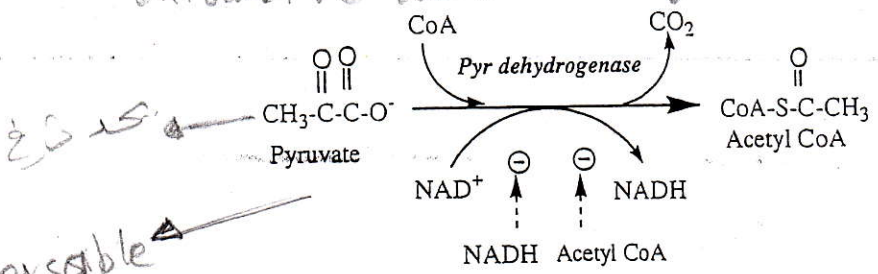


Figure ( ) Oxidative decarboxylation of pyruvate.

Pyruvate dehydrogenase complex is a multienzyme complex of three enzymes: *pyruvate dehydrogenase*, *dihydrolipoyl transacetylase*, and *dihydrolipoyl dehydrgenase*. *Pyruvate dehydrogenase complex* contains five coenzymes that act as carriers or oxidants for the intermediates of the reaction. The enzyme is inhibited by acetyl CoA, which accumulated when its produced faster than it can be oxidized by the TCA-cycle. The enzyme is also inhibited by elevated levels of NADH.

Regulation of Glycolysis:

The ***PFK-1*** reaction is the rate-limiting step in glycolysis. It is inhibited allosterically by elevated levels of **ATP**, which act as an "energy-rich" signal indicating an abundance of high-energy compounds. Elevated levels of **citrate** also inhibit *PFK-1*. Conversely, *PFK-1* is activated allosterically by high concentrations of **AMP**, which signal that the cell's energy sources are depleted. **Fructose-2,6-bisphosphate** is the most potent activator of *PFK-1*. Decreased levels of glucagons and elevated levels of insulin, such as occur following a carbohydrate-rich meal, cause an increase in fructose-2,6-bisphosphate and in the rate of glycolysis. Fructose-2,6-bisphosphate thus acts as an intracellular signal, indicating that glucose is abundant.

In liver, ***pyruvate kinase*** is activated by fructose-1,6-bisphosphate, the product of the *PFK-1* reaction. This feed-forward regulation has the effect of linking the two kinase activities: increased *PFK-1* activity, resulting in elevated levels of fructose-1,6-bisphosphate, activates *pyruvate kinase*.

Phosphorylation by a *cAMP-dependent protein kinase* leads to inactivation of ***pyruvate kinase*** in the liver. When blood glucose levels are low, elevated glucagons increases the intracellular

level of cAMP, which favors the phosphorylation and inactivation of *pyruvate kinase*. Therefore, PEP is unable to continue in glycolysis, but instead enters the gluconeogenesis pathway. This in part explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagons. Dephosphorylation by a ***phosphoprotein phosphatase*** results in reactivation of the enzyme.

Clinical Aspects:

1) *Pyruvate kinase* deficiency:

Genetic deficiency of *pyruvate kinase* (*PK*) in the erythrocyte leads to hemolytic anemia. The normal mature erythrocyte lacks mitochondria and is completely dependent on glycolysis for its production of ATP. The anemia observed in *PK* deficiency is a consequence of the reduced rate of glycolysis and the rate of ATP synthesis being inadequate to meet the energy needs of the cell and maintain the structural integrity of the erythrocyte membrane. The alterations in the red blood cell membrane lead to changes in the shape of the cell and ultimately to phagocytosis by the cells of the reticuloendothelial system, particularly macrophages of the spleen. The premature death and lyses of the red blood cell results in hemolytic anemia.

2) Inherited deficiency of *pyruvate dehydrogenase*: This has been reported. Lactic acidosis is seen (specially after glucose load).

3) Mercuric (Hg^{2+}) ions can complex with the —SH group of lipoic acid and inhibits *pyruvate dehydrogenase complex.*

4) Dietary deficiencies of vitamin B_1 (Thiamine): Has similar effect. Lack of TPP inhibits *pyruvate dehydrogenase*, leading to accumulation of pyruvate and lactate.

5) Alcoholism: Chronic alcoholics suffer from nutritional deficiency of vitamin B_1 (thiamine) which will has similar effect, resulting to accumulation of pyruvate and lactate.

6) Sodium fluoride is used for collection of blood for glucose estimation. Fluoride has the following functions:

i) inhibits in vitro glycolysis by inhibiting enzyme *enolase.*

ii) acts as anticoagulant.

iii) antiseptic.

CITRIC ACID CYCLE

The citric acid cycle also called the Krebs cycle or the tricarboxylic acid –TCA-cycle. Its central function is the oxidation of acetyl-CoA to CO_2 and H_2O. Acetyl CoA is derived from the metabolism of fuel molecules such as amino acids, fatty acids, and carbohydrates. The TCA-cycle also participates in a number of synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of amino acids and provides building blocks for heme synthesis. The cycle occurs totally in the mitochondrial matrix and is therefore in close proximity to reactions of

oxidative phosphorylation. In the cycle oxaloacetate is first condensed with acetate (acetyl CoA), then regenerated as the cycle is completed.

Reactions of the TCA-cycle:

The cycle is started by the condensation of acetyl CoA and oxaloacetate to citrate by ***citrate synthase***. Citrate is isomerized to isocitrate by ***aconitase***. Isocitrate is oxidatively decarboxylated to α-ketoglutarate by ***isocitrate dehydrogenase***, yielding the first of the three NADH molecules produced by the cycle, and the first release of CO_2. Conversion of α-ketoglutarate to succinyl CoA is catalyzed by the ***α-ketoglutarate dehydrogenase complex***. The reaction releases the second CO_2 and produces the second NADH of the cycle. ***Succinate thiokinase*** cleaves the high-energy thioester bond of succinyl CoA to succinate. This reaction is coupled to phosphorylation of GDP to GTP (substrate-level phosphorylation). Succinate is oxidized to fumarate by ***succinate dehydrogenase***, producing the reduced coenzyme $FADH_2$. Fumarate is hydrated to malate in a reaction catalyzed by ***fumarase***. Malate is oxidized to oxaloacetate by ***malate dehydrogenase***. This reaction produces the third and final NADH of the cycle.

The whole process is aerobic, requiring O_2, as the final oxidant of the reducing equivalents. Absence of O_2 (anoxia) or partially deficiency of O_2 (hypoxia) causes total or partial inhibition of the cycle.

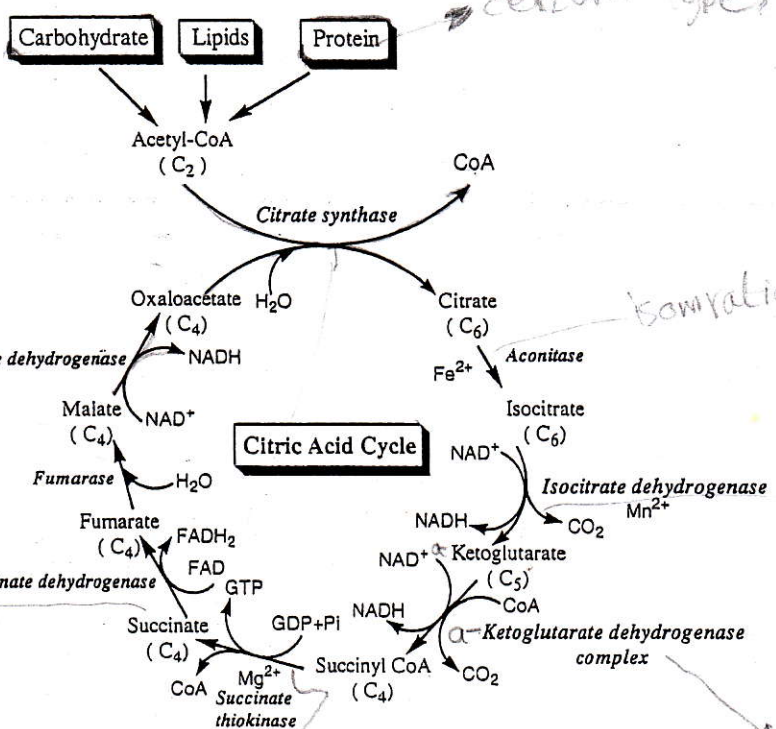


Figure: Krebs cycle.

Regulation of the TCA-cycle:

The most important regulatory enzymes in this cycle are ***citrate synthase***, ***isocitrate dehydrogenase***, and ***α-ketoglutarate dehydrogenase complex***.

Citrate synthase is inhibited by ATP, NADH, succinyl CoA, and acyl CoA derivatives of fatty acids.

Citrate also inhibits *phosphfructokinase* (the rate-setting enzyme of glycolysis), and activates *acetyl CoA carboxylase* (the rate-limiting enzyme of fatty acid synthesis).

Isocitrate dehydrogenase is activated by ADP and inhibited by ATP and NADH.

α-ketoglutarate dehydrogenase is inhibited by ATP, GTP, NADH and succinyl CoA.

TCA-cycle is Amphibolic in Nature:

TCA-cycle has dual role:

1) Catabolic Role:

The two carbon compound acetyl CoA is oxidized in this cycle to produce CO_2, H_2O, and energy as ATP.

2) Anabolic Role:

Intermediates of TCA-cycle are utilized for synthesis of various compounds.

i) Heme Synthesis:

Succinyl CoA is used in the biosynthesis of heme.

Succinyl CoA + Glycine $\xrightarrow{Fe^{++}}$ Porphyrin $\longrightarrow$ Heme

ii) Fatty Acid Synthesis:

Citrate provides a source of acetyl CoA for the cytosolic synthesis of fatty acids, and also for synthesis of cholesterol and steroids.

iii) Synthesis of Non-essential Amino Acids:

The conversion of pyruvate to alanine, oxaloacetate to aspartate, and α-ketoglutarate to glutamate is catalyzed by ***transaminase***. Because these reactions are reversible, the TCA-cycle serves as a source of carbon skeleton for the synthesis of non-essential amino acids.

iv) Gluconeogenesis.

GLUCONEOGENESIS

Gluconeogenesis means the formation of glucose from non-carbohydrate sources. Glyerol, lactate, and the glucogenic amino acids are the most important gluconeogenic precursors.

Gluconeogenesis is found in the liver and kidney only. Approximately 90 % of gluconeogenesis occurs in the liver, whereas kidneys provide 10 % of newly synthesized glucose molecules.

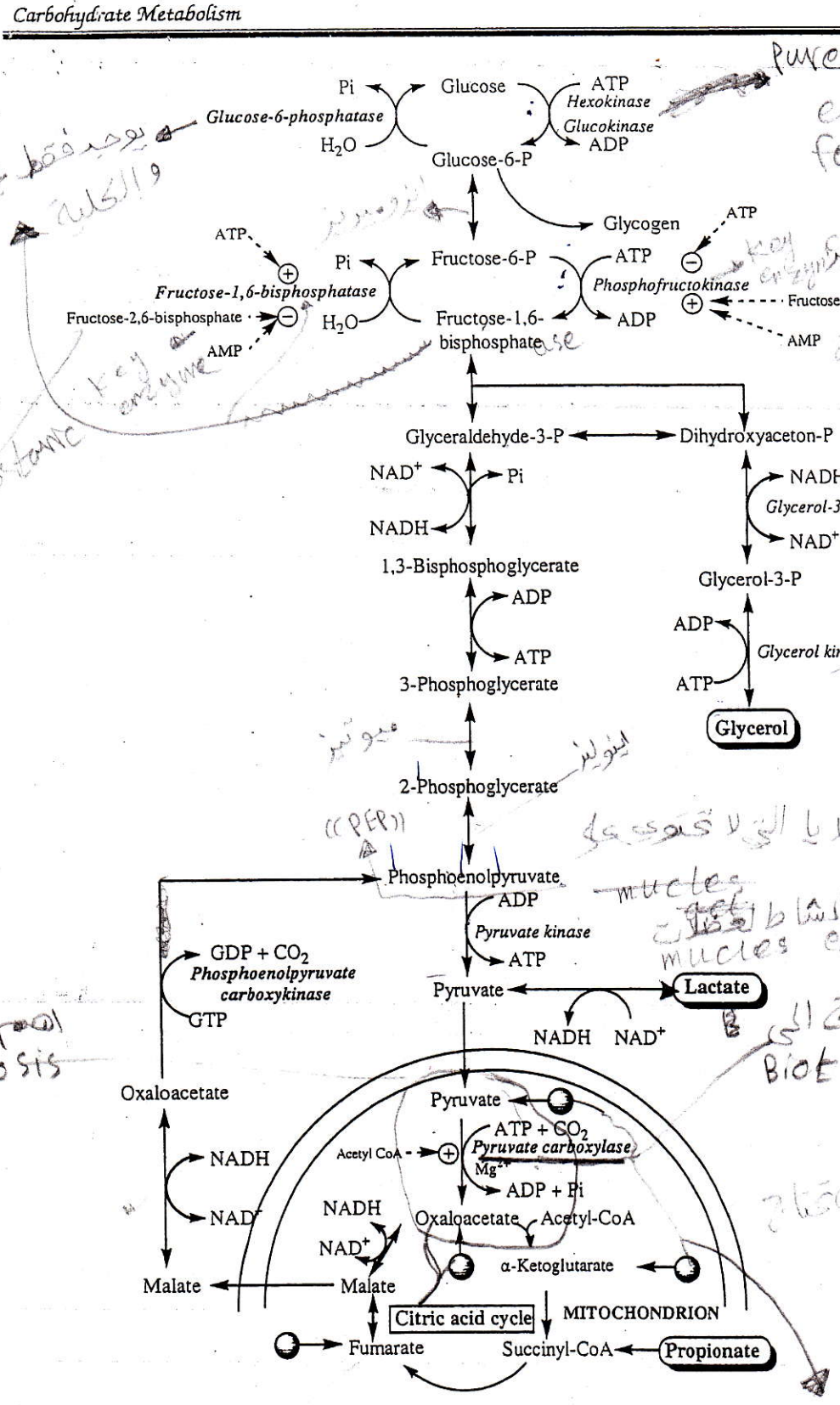


Figure: Gluconeogenesis Pathway.

In glycolysis, glucose is converted into pyruvate, whereas in gluconeogenesis pyruvate is converted to glucose. But, gluconeogenesis is not a simple reversal of glycolysis, due to the presence of 3-energy barriers which are irreversible.

Reactions of the Gluconeogenesis:

Pyruvate is first carboxylated by ***pyruvate carboxylase*** to oxaloacetate. *Pyruvate carboxylase* is found in the mitochondria of liver and kidney cells, but not of muscles. Oxaloacetate, formed in mitochondria, must enter the cytosol where the other enzymes of gluconeogenesis are located. However, oxaloacetate is unable to cross the inner mitochondrial membrane directly; it must first be reduced to malate, which can be transported from the mitochondria to the cytosol.

In the cytosol, malate is reoxidized to oxaloacetate, which is decarboxylated and phosphorylated to phosphoenolpyruvate (PEP) by ***PEP-carboxykinase***. The reaction is driven by hydrolysis of GTP. PEP is then enters the reversed reactions of glycolysis until it reaches fructose-1,6-bisphosphate. ***Fructose-1,6-bisphosphatase*** hydrolyzes fructose-1,6-bisphosphate to fructose-6-phosphate. This reaction is an important regulatory site of gluconeogenesis. *Fructose-1,6-bisphosphatase* occurs in liver and kidney. Finally glucose-6-p hydrolyzed to glucose by ***glucose-6-phosphatase***. *Glucose-6-phosphatase* occurs in liver and kidney, but not in muscles. Thus, muscle cannot provide blood glucose by gluconeogenesis.

Gluconeogenic Precursors:

1. **Glycerol** is released during hydrolysis of triacylglycerols in adipose tissue and is delivered by the blood to the liver. Glycerol is phosphorylated to glycerol-3-P, which is oxidized to dihydroxyacetone phosphate, an intermediate of glycolysis.
2. **Lactate** is released into the blood as a result of muscle exercise from glycolysis. This lactate is taken up by the liver and converted to glucose, which is released back into the circulation. This cycle is called **Cori cycle.**
3. **α-Ketoacids**, such as pyruvate, oxaloacetate, and α-ketoglutarate, are derived from the metabolism of glucogenic amino acids. These substances can enter the TCA-cycle and form oxaloacetate.

Regulation of the Gluconeogenesis:

Fructose-1,6-bisphosphatase is inhibited by elevated levels of AMP, which signal an "energy-poor" state in the cell. Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis.

The regulation of gluconeogenesis is determined primarily by the circulating level of glucagon and by availability of gluconeogenic substrates.

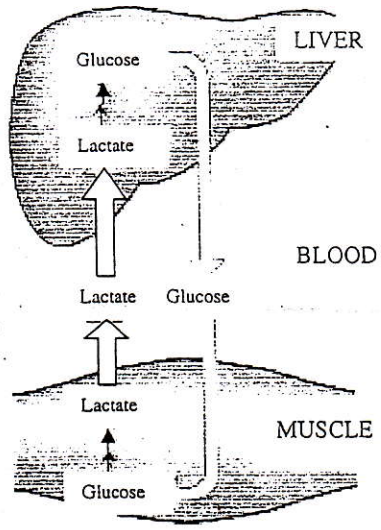


Figure: The Cori cycle.

Glucagon:

Glucagon stimulates gluconeogenesis by two mechanisms:

1. **Changes in allosteric effectors:** Glucagon lowers the level of fructose-2,6-bisphosphate, resulting in activation of *fructose-1,6-bisphosphatase* and inhibition of *phosphofructokinase*.
2. **Covalent modification of enzyme activity:** Glucagon, via an elevation in cAMP level and *cAMP-dependent protein kinase* activity, stimulates the conversion of *pyruvate kinase* to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose.

Substrate availability:

Decreased levels of insulin favour mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis.

Allosteric activation by acetyl CoA:

Allosteric activation of hepatic *pyruvate carboxylase* by acetyl CoA occurs during starvation. As a result of excessive lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O. As a result, acetyl CoA accumulates and leads to activation of *pyruvate carboxylase*.

The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of each molecule of glucose.

oxidation is the first step
specilize tissus
Coenzym needed NADP

HEXOSE MONOPHOSPHATE PATHWAY

The hexose monophosphate pathway (HMP) also called pentose phosphate pathway, phosphogluconate pathway, Warburg-Dickens-Lipman pathway. The HMP occurs in the cytosol of the cell. No ATP is directly consumed or produced in the cycle.

The HMP is particularly important in liver and mammary glands, which are active in the biosynthesis of fatty acids, and in the adrenal cortex, which is active in the NADPH-dependent synthesis of steroids.

The sequence of reactions of the pathway may be divided into 2-phases, oxidative and nonoxidative. The oxidative portion of the HMP consists of three reactions that lead to the formation of ribulose-5-phosphate, CO_2 , and two molecules of NADPH for each molecule of glucose-6-P oxidized.

The nonoxidative reactions of the HMP permit ribulose-5-phosphate (produced by the oxidative portion of the pathway) to be converted either to ribose-5-phosphate (needed for nucleotide synthesis) or to intermediates of glycolysis, such as fructose-6-P and glyceraldehyde-3-phosphate.

The pathway starts with the dehydrogenation of glucose-6-P to 6-phosphogluconolactone by *glucose-6-P dehydrogenase* which is a $NADP^+$-dependent enzyme. This reaction is the rate-regulatory step in the pathway. The enzyme being inhibited readily by the product NADPH. The rise in NADPH level inhibits both *glucose-6-P dehydrogenase* and *6-phosphogluconate dehydrogenase*. Activities of both dehydrogenases and the rate of the pathway are enhanced on feeding high carbohydrate diets and are reduced in starvation and diabets mellitus.

Transketolase transfers the 2-carbon unit comprising carbons 1 and 2 of a ketose to the aldehyde carbon of an aldose sugar.

Transaldolase allows the transfer of a 3-carbon moiety (carbons 1, 2, 3) from the ketose sedoheptulose-7-P to the aldose glyceraldehyde-3-P.

For one round of the HMP, 3 molecules of glucose are required to produce 2 molecules of fructose-6-P and 1 molecule of glyceraldehyde-3-P and 6 NADPH molecules.

Metabolic significance of HMP shunt:

1) **Formation of NADPH:** This pathway provides a major portion of the cell's NADPH, which functions as a biochemical reductant. THF فيتامين
2) **Provision of pentoses:** Produces ribose-phosphate, required for biosynthesis of nucleotides, and provides a mechanism for the metabolic utilization of five-carbon sugars ingested as food.

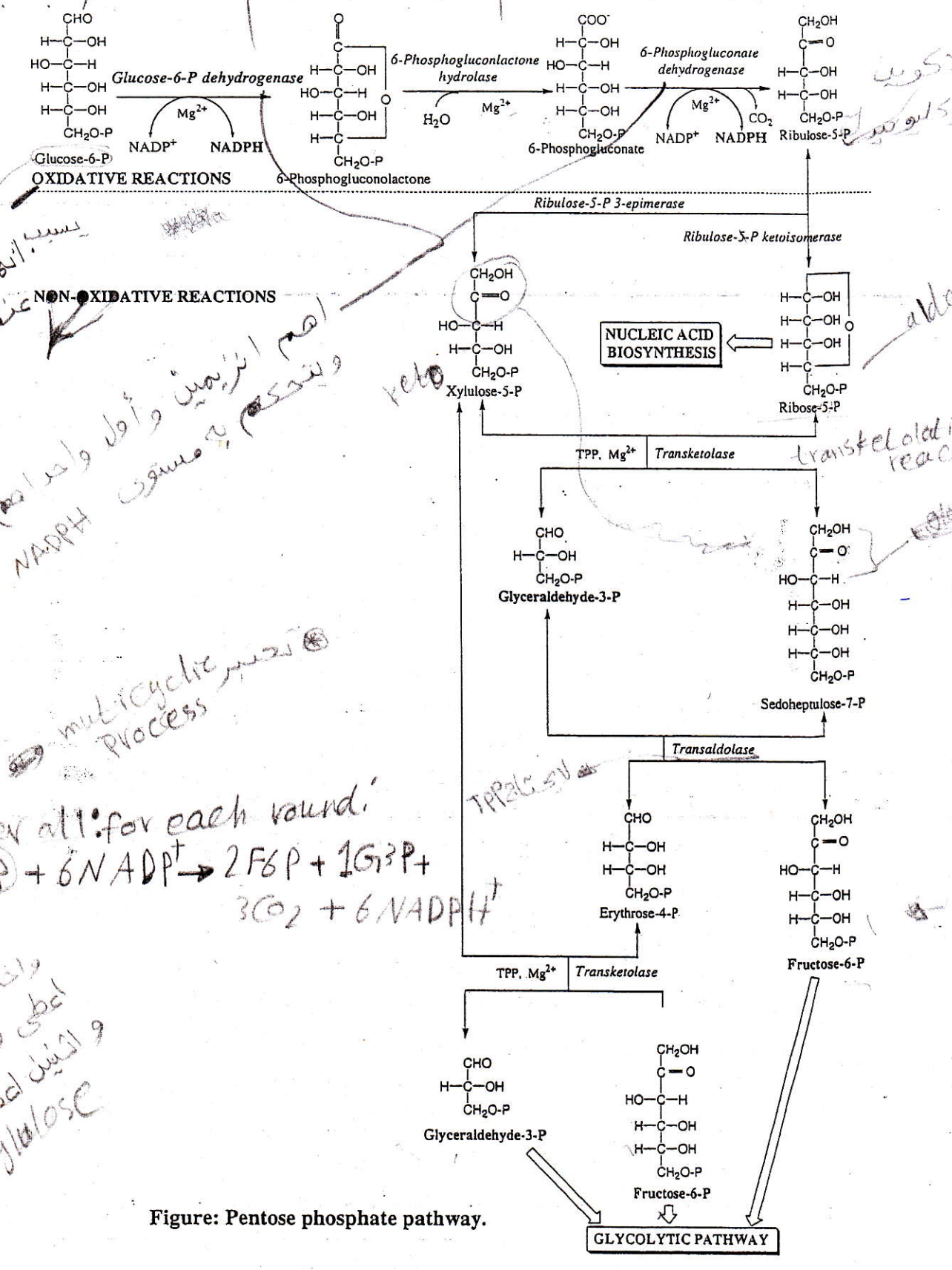


Figure: Pentose phosphate pathway.

3) **Supply of arabinose-5-P:** Arabinose-5-P can be produced, as discussed above, and is used for incorporating arabinose in glycoproteins.

Ribose-5-P —(*Phosphopentose-2-epimerase*)→ Arabinose-5-P

4) **Role in lens metabolism:** In studing lens metabolism, it has been observed, at least 10% of glucose is metabolized by shunt pathway and provides NADPH, which is necessary to convert oxidized glutathione, which is necessary for maintenance of lens proteins.

5) **Role in phagocytosis:** Reactions of this pathway are increased mainfold in leucocytes during "phagocytosis". After phagocytosis has occurred, *NADPH oxidase*, located in the leukocyte cell membrane, converts molecular oxygen O_2 from the surrounding tissue into superoxide O_2^-. Next, superoxide is converted into H_2O_2 by *superoxide dismutase* (*SOD*). In the presence of *oxygen-dependent myeloperoxidase* (*MPO*), a lysosomal enzyme present within the phagolysosome, peroxide plus chloride ions (Cl^-) are converted into hypochlorous acid (HOCl, the major component of household bleach), that kills the bacteria. Excess peroxide is either neutralized by *catalase* or by *glutathione peroxidase*.

6) **Role in tissue anoxia:** Tissues subjected to extended periods of anoxia develop fatty infiltration. Increased amounts of glucose may be metabolized by way of HMP-shunt, in situations resulting from tissue anoxia. The mechanism involved appears to be that the lack of tissue O_2 decreases the metabolism of pyruvate by way of TCA cycle. Intermediates of anaerobic glycolysis accumulate resulting in a diversion of glucose-6-P into HMP-shunt pathway, resulting to increased amount of NADPH. The excess NADPH is diverted to increased fatty acid synthesis, thus accounting for "fatty infiltration".

7) **Role in erythrocytes fragility:** HMP-shunt in erythrocytes provides NADPH for:

i) reduction of oxidized glutathion (G-S-S-G) to reduced glutathion (2 G-SH) catalyzed by the enzyme *glutathione reductase*.

ii) reduced glutathion (2 G-SH) thus formed in turn removes hydrogen peroxide (H_2O_2) from the erythrocytes in a reaction catalyzed by *glutathione peroxidase*.

$$2\,G\text{-}SH + H_2O_2 \xrightarrow[\text{Glutathione reductase}]{NADP^+ \rightarrow NADPH} G\text{-}S\text{-}S\text{-}G + H_2O \quad (\text{Glutathione peroxidase})$$

This reaction is important, since accumulation of H_2O_2 may decrease the life span of RBCs by increasing the rate of oxidation of Hb to methemoglobin.

An inverse correlation exists between the activity of *glucose-6-P dehydrogenase* and the fragility of red blood cells.

Reduction of hydrogen peroxide:

Hydrogen peroxide is one of a family of reactive oxygen intermediates that are formed from the partial reduction of molecular oxygen (O_2). These compounds are formed continuously as by-products of aerobic metabolism and through reactions with drugs and environmental toxins. They are highly reactive and can cause serious chemical damage to DNA, proteins, and unsaturated lipids. Reactive oxygen intermediates have been implicated in a number of pathological processes, including reperfusion injury, cancer, inflammatory disease, and aging. The cell has several protective mechanisms that serve to minimize the toxic potential of these compounds.

Reduced glutathione (2G-SH) present in most cells can chemically detoxify H_2O_2. This reaction catalyzed by *glutathione peroxidase*, forms oxidized glutathione (G-S-S-G), which no longer has protective properties. The cell regenerates reduced glutathione in a reaction catalyzed by *glutathione reductase* using NADPH as a source of reducing electrons. Thus, NADPH indirectly provides electrons for the reduction of H_2O_2.

A number of intracellular reducing agents, such as ascorbate, vitamin E, and β-carotene, are able to reduce and, thus, detoxify oxygen intermediates in cells. Consumption of foods rich in these "antioxidant" compounds has been correlated with a reduced risk for certain types of cancers as well as decreased frequency of certain other chronic health problems.

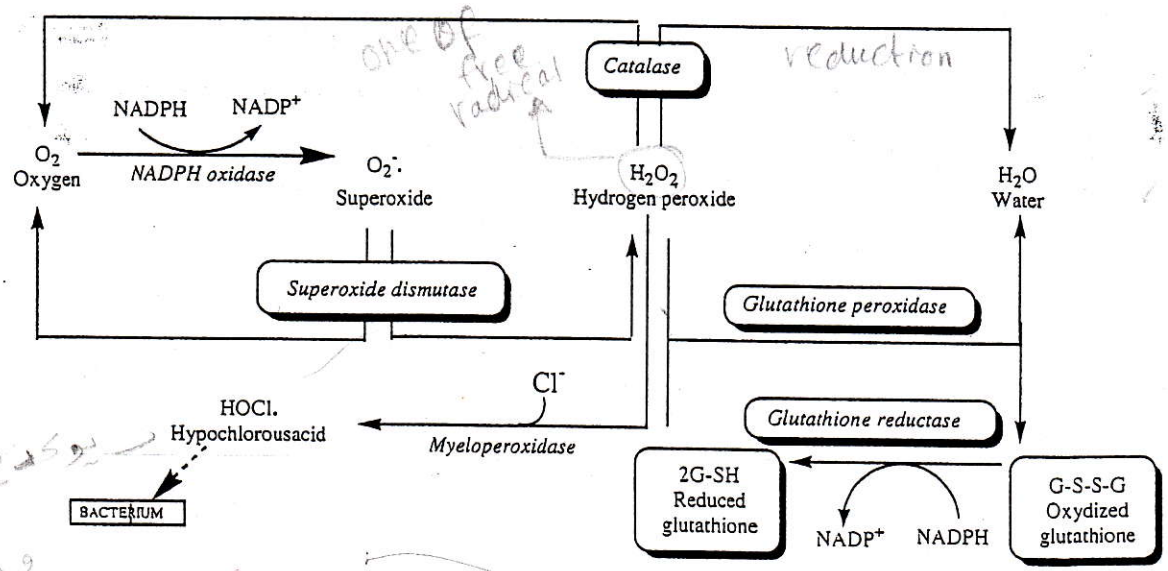


Figure: Formation of reactive intermediates from molecular oxygen, and actions of antioxidant enzymes.

***Glucose-6-P dehydrogenase* deficiency:**

Glucose-6-P dehydrogenase deficiency is an inherited disease characterized by hemolytic anemia caused by the inability to detoxify oxidizing agents. Diminished *Glucose-6-P dehydrogenase* activity impairs the ability to form NADPH that is essential in the detoxification of free radicals and peroxides formed within the cell. Although the deficiency occurs in all cells of the infected individual, it is more sever in erythrocytes where the HMP provides the only means of generating NADPH.

The major role of NADPH in erythrocytes is to reduce the disulfide (-S-S-) of glutathione to the sulfhydryl form (-SH HS-). This reaction is catalyzed by *glutathione reductase*. The reduced form of glutathione to maintains the cysteine residues of Hb and other erythrocyte proteins in the reduced state. The reduced form also plays a role in detoxification by reacting with H_2O_2 and free radicals. Reduced glutathione appears to be essential for maintaining normal erythrocyte structure and for keeping hemoglobin in the ferrous (Fe^{++}) state. Cells with lowered level of reduced glutathione are more susceptible to hemolysis.

$$\text{Glucose-6-P} + \text{NADP}^+ \xrightarrow{\textit{Glucose-6-P dehydrogenase}} \text{6-Phosphogluconate} + \text{NADPH}$$

$$\text{G-S-S-G} + \text{NADPH} \xrightarrow{\textit{Glutathione reductase}} 2\,\text{G-SH} + \text{NADP}^+$$

$$H_2O_2 + 2\,\text{G-SH} \xrightarrow{\textit{Glutathione peroxidase}} H_2O + \text{G-S-S-G}$$

$$\text{RBC} + \text{Oxidant} \longrightarrow H_2O_2$$

Patients with *glucose-6-P dehydrogenase* deficiency develop hemolytic anemia if they are treated with an oxidant drug, i.e. antibiotics (for example sulfamethoxazole), antimalarials (for example, primaquine) and antipyretics (for example, acetanilide, but not aspirin or acetaminophen).

GLYCOGEN METABOLISM

Liver and skeletal muscles are the main sites for storage of glycogen in human. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of ATP during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast. Approximately 400 g of glycogen makes up 1 % to 2 % of the fresh weight of resting muscle, and approximately 100 g of glycogen makes up 6 % to 8 % of the fresh weight of a well-fed adult liver.

GLYCOGENESIS:

The formation of glycogen from glucose. It is a cytosolic process occurs principally in liver and skeletal muscles, but it can occur in every tissue to some extent.

The process is started by phosphorylation of glucose to glucose-6-P as in glycolysis. This reaction is catalyzed by *hexokinase* in muscles and *glucokinase* in liver. Glucose-6-P is converted to glucose-1-P by *phosphoglucomutase*. Glucose-1-P reacts with UTP to form the activated glucose (UDP-Glucose), the reaction is catalyzed by *UDP-Glucose pyrophosphorylase*.

Glycogen synthase is responsible for making the α-1,4 linkages in glycogen This enzyme cannot initiate chain synthesis using free glucose as an acceptor of a molecule of glucose from UDP-Glucose. A fragment of glycogen can serve as a primer. In the absence of a glycogen primer, a specific protein, called **glycogenin**, can serve as an acceptor of glucose residues. By the action of ***glycogen synthase*** the C_1 of the UDP-Glucose forms α-1,4 glycosidic bond with the C_4 of the terminal glucose residue of the primer, liberating the UDP. Elongation of glycogen involves the transfer of glucose from UDP-Glucose to the nonreducing end of the growing chain. Branches are made by the action of the ***branching enzyme***. This enzyme transfers a chain of 5 to 8 glycosyl residues from the nonreducing end of the glycogen chain (breaking an α-1,4 bond) to another residue on the chain and attaches it by an α-1,6 bond. The resulting new, nonreducing end as well as the old nonreducing end from which the 5 to 8 residues were removed be further elongated by ***glycogen synthase***.

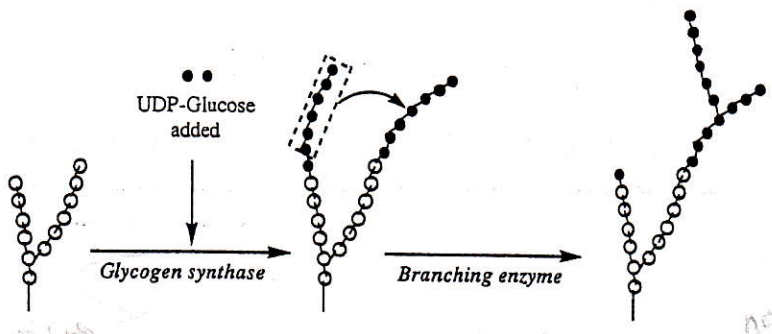


Figure: Steps of glycogenesis.

GLYCOGENOLYSIS:

The degradation of glycogen to glucose.

The first step is the action of ***glycogen phosphorylase***, which cleaves the α-1,4 glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis (to form glucose-1-P) until 4 glucosyl units remain on each chain before a branch point. The resulting structure is called a limit dextrin, and *phosphorylase* cannot degrade it any further. *Oligo-(α-1,4→ α-1,4)- glucantransferase* removes the outer three of the four glucosyl residues attached at a branch and transfers them to the nonreducing end of another chain, lengthening

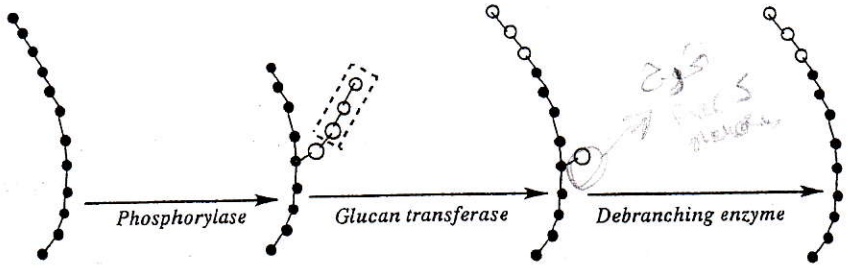


Figure : Steps of glycogenolysis.

it accordingly. The remaining single glucose residue attached in an α-1,6 linkage is removed hydrolytically by the *amylo-α-(1,6)-glucosidase* activity (***debranching enzyme***), releasing free glucose. The glucosyl chain is now available again for degradation by ***glycogen phosphorylase*** until 4 glucosyl units from the next branch are reached.

Glucose-1-P, produced by *glycogen phosphorylase*, is converted to glucose-6-P by *phosphoglucomutase*. In liver, a specific enzyme ***glucose-6-phosphatase*** converts glucose-6-P to free glucose which diffuses to the blood. In skeletal muscles, *glucose-6-phosphatase* is absent. Hence glucose-6-P enters in glycolytic pathway and forms pyruvate and finally lactic acid. Muscle glycogenolysis does not contribute to blood glucose directly. But indirectly, lactic acid is converted to glucose via Cori cycle in liver.

Regulation of Glycogenesis & Glycogenolysis:

Glycogen phosphorylase and ***glycogen synthase*** are the regulatory enzymes in glycogenolysis and glycogenesis respectively. These enzymes are regulated by a complex series of reactions involving both allosteric mechanisms and covalent modifications due to phosphorylation and dephosphorylation of enzymes.

When blood glucose level is decreased, hormones such as glucagon and epinephrine are released. The binding of the hormone glucagon or epinephrine to the hepatocyte receptors, or epinephrine to muscle cell receptors, results in the activation of ***adenylate cyclase***. This enzyme catalyzes the synthesis of cAMP, which activates ***cAMP-dependent protein kinase***. This enzyme is a tetramer, having two regulatory subunits (R) and two catalytic subunits (C). cAMP binds to the regulatory subunit dimmer, releasing individual catalytic subunits that are active. Active *cAMP-dependent protein kinase* phosphorylates the inactive form of ***phosphorylase kinase***, resulting in its activation. Active *phosphorylase kinase* phosphorylates *glycogen phosphorylase* (inactive), converting it into active ***glycogen phosphorylase***, which is then begins glycogen breakdown.

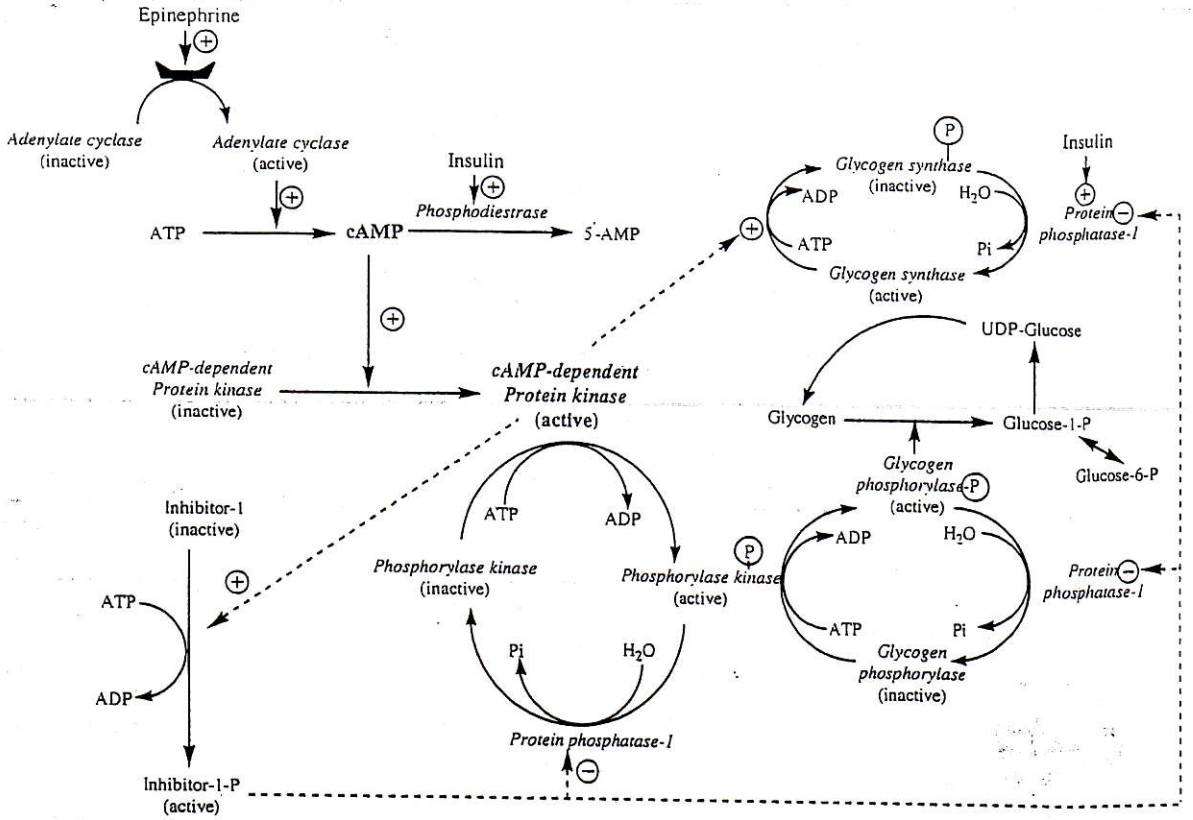


Figure: Regulation Of Glycogenesis & Glycogenolysis

At the same time active *cAMP-dependent protein kinase* phosphorylates and thereby inactivates ***glycogen synthase*** (active form). Also active *cAMP-dependent protein kinase* phosphorylates the protein factor called **inhibitor-1** (inactive) and converts it to active **inhibitor-1-P**, which inhibits ***protein phosphatase-1*** (which removes the phosphate groups hydrolytically), that in turn inhibits the conversion of the active *phosphorylase kinase* to the inactive form of the enzyme, the active *glycogen phosphorylase* to the inactive form of the enzyme, and also inhibits the conversion of ***glycogen synthase*** (incative) to the active form of the enzyme. The result of all these reactions is the activation of glycogenolysis and inhibition of glycogenesis.

When blood glucose level is increased, insulin released. This hormone stimulates glycogenesis via increasing the activity of ***protein phosphatase-1*** and also activates ***phosphodiestrase*** which reduces the level of cAMP by converting it to AMP the result is the inactivation of *cAMP-dependent protein kinase.*

In the well-fed state, ***glycogen synthase*** is allosterically activated by glucose-6-P when it is present in elevated concentrations. In contrast, ***glycogen phosphorylase*** is allosterically inhibited by glucose-6-P, as well as by ATP. In liver, glucose also serves as an allosteric inhibitor of *glycogen phosphorylase*. During muscle contraction, Ca^{2+} binds to **calmodulin** - a subunit of *phosphorylase*

kinase- and activates this enzyme. High concentrations of AMP activates muscle ***glycogen phosphorylase*** and therefore activates glycogenolysis.

METABOLISM OF MONOSACCHARIDES

Fructose and galactose are two monosaccharides other than glucose occur in significant amounts in the diet and make important contributions to energy metabolism. In addition, galactose is an important component of cell structural carbohydrates.

Fructose Metabolism:

Entry of fructose into cells is not insulin dependent. For fructose to enter the pathways of intermediary metabolism, it must first be phosphorylated. This can be accomplished by either *hexokinase* or *fructokinase*. ***Hexokinase*** phosphorylates glucose in all cells of the body. ***Fructokinase*** provides the primary mechanism for fructose phosphorylation. It is found in the liver (which processes most of the dietary fructose), kidney, and the small intestine, and converts fructose to fructose-1-P using ATP as the phosphate donor. Fructose-1-P is cleaved by ***aldolase B*** into dihydroxyaceton phosphate (DHAP) and D-glyceraldehyde. DHAP can directly enter glycolysis or gluconeogenesis, whereas D-glyceraldehyde can be metabolized by a number of pathways, as illustrated in **Figure** . [Note: *Aldolase A* primarily cleaves fructose-1,6-bisphosphate produced during glycolysis to DHAP and glyceraldehydes-3-P].

The rate of fructose metabolism is more rapid than that of glucose because the trioses formed from fructose-1-P bypass *phosphofructokinase*, the major rate-limiting step in glycolysis. Elevated levels of dietary fructose significantly elevate the rate of lipogenesis in the liver, owing to the rapid production of acetyl CoA. This may causes atherosclerosis.

Clinical Aspects:

1. **High-fructose diets:**

Excessive fructose consumption can adversely affect liver metabolism. The phosphorylation of fructose to fructose-1-P is rapid, whereas the *aldolase B* reaction is relatively slow. As a result, fructose-1-P may accumulate, with an accompanying decrease in intracellular inorganic phosphate (Pi) level. [Note: This is referred to as "sequestering of phosphate", where phosphate is attached covalently to an organic molecule and therefore is no longer available to participate in other essential metabolic reactions.] The lowered availability of Pi, therefore, limits the rate of production of ATP from ADP + Pi, especially in the liver, which metabolizes most dietary fructose. The resulting ADP (and AMP) are sequently catabolized, leading to hyperuricemia and gout.

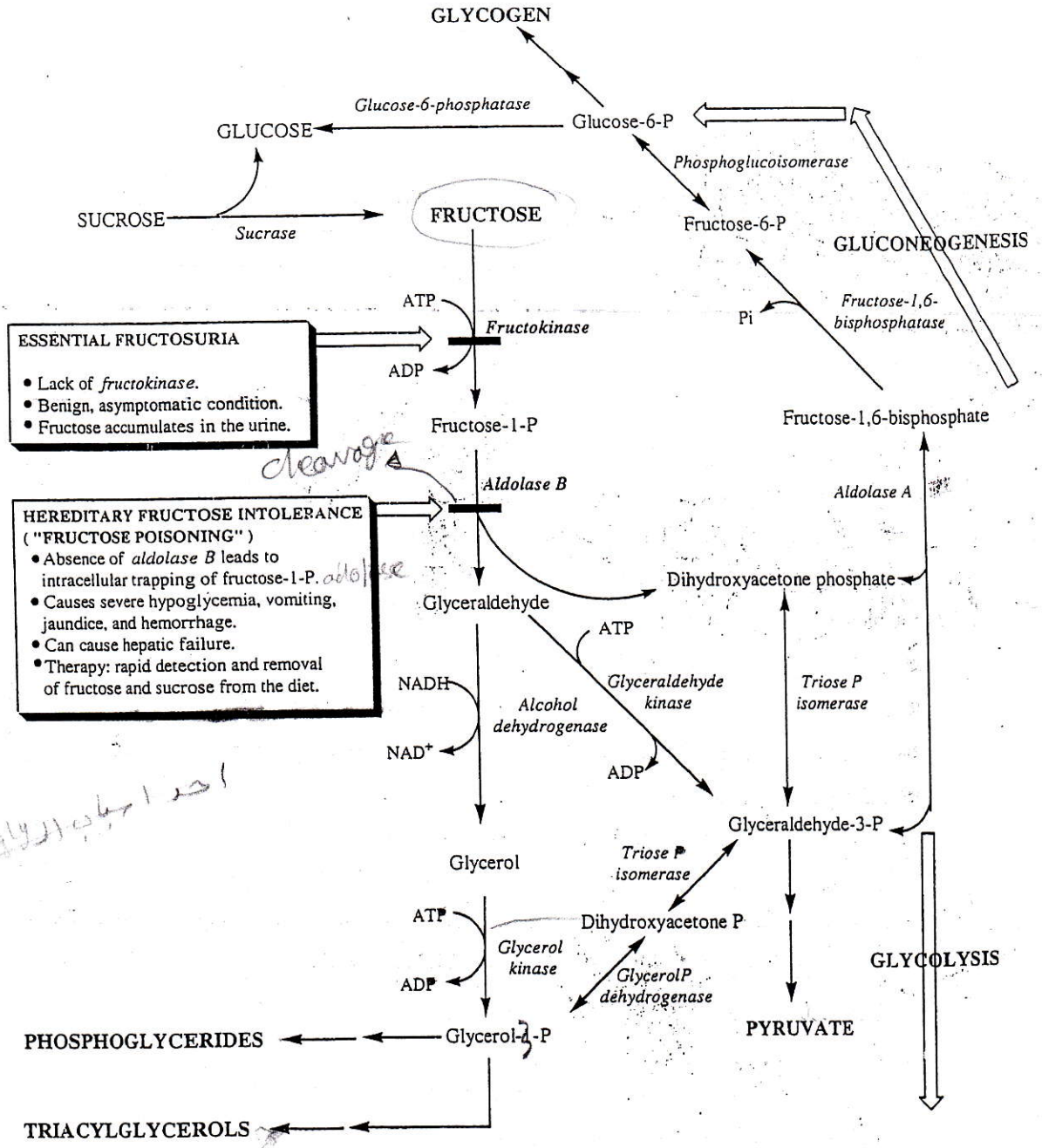


Figure: Fructose metabolism.

2. Genetic diseases:

A deficiency or absence of ***aldolase B*** leads to the accumulation of fructose-1-P, which develops hypoglycemia. In this case, however, the hypoglycemia is produced not by excess insulin but by the accumulation of fructose-1-P in the liver. This compound is an inhibitor of ***aldolase A***. Thus, glucose is not catabolized. Even more important is the fact that this same enzyme also catalyzes the synthesis of glucose (gluconeogenesis). Therefore, the inhibition of the enzyme lowers the glucose contents in the blood while it rises the fructose content. Furthermore, the blood Pi content is also

lowered because the accumulating fructose-1-P ties up the free Pi. This accumulation in the liver also produces liver enlargement and jaundice. The disease can be treated by removing fructose and sucrose from the diet.

3. Conversion of glucose to fructose by way of sorbitol:

self study

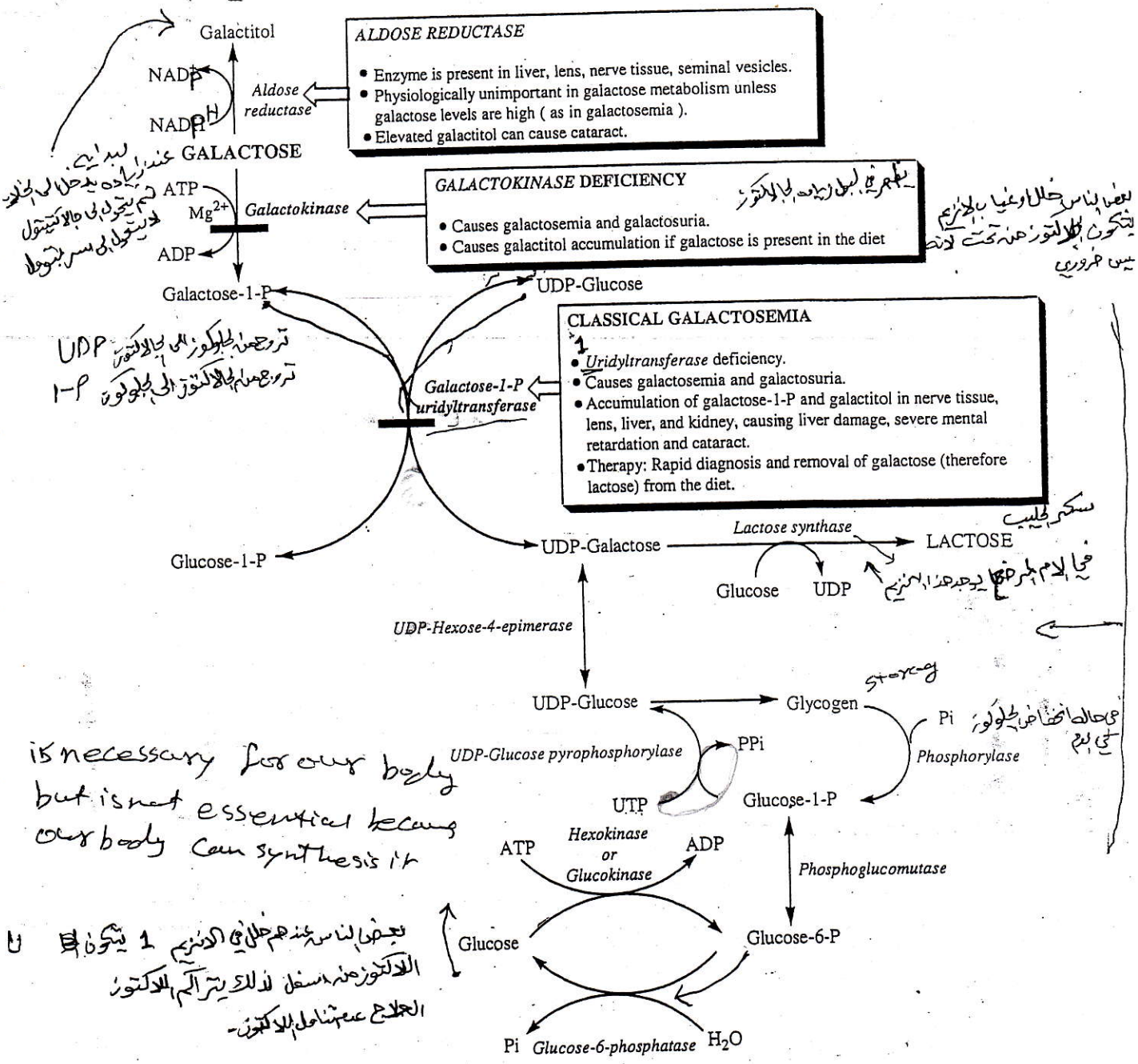
In many tissues such as the lens, retina, Schwan cell of peripheral nerves, kidney, placenta, RBC_s, and in cells of ovaries and seminal vesicles glucose is reduced to sorbitol by ***aldose reductase***. In the liver, ovaries, sperm, and seminal vesicle cells there is a second enzyme, ***sorbitol dehydrogenase*** that can oxidize the sorbitol to produce fructose. The two-reaction pathway from glucose to fructose in the seminal vesicles is for sperm cells, for which fructose is a preferred carbohydrate energy source. Because insulin is not required for entry of glucose into the cells listed above, large amounts of glucose may enter these cells during times of hyperglycemia, for example, in uncontrolled diabetes. Elevated intracellular glucose concentrations, and an adequate supply of NADPH, cause *aldose reductase* to produce a significant amount of sorbitol, which, unlike glucose, cannot pass efficiently through cell membranes and therefore remains trapped inside the cell. This is exacerbated when *sorbitol dehydrogenase* is low or absent, for example, in retina, lens, kidney, and nerve cells. As a result, sorbitol accumulates in these cells, causing strong osmotic effects and therefore cell swelling due to water retention. Some of the pathologic alterations associated with diabetes can be attributed to this phenomenon, including cataract formation, peripheral neuropathy, and vascular problems leading to nephropathy and retinopathy.

Galactose Metabolism:

Like fructose, entry of galactose into cells is not insulin dependent. Galactose is readily converted in the liver to glucose, as in Figure .

Glucose-1-P-galactose-1-P uridyltransferase is missing in individuals with classical galactosemia. If this enzyme is missing, galactose-1-P and, therefore, galactose, accumulates in cells. Physiologic consequences are similar to those found in essential fructose intolerance, but a wider spectrum of tissues is affected. The accumulated galactose is shunted into side pathway such as that of galactitol production. This reaction is catalyzed by the same enzyme, ***aldose reductase*** that converts glucose to sorbitol.

UDP-galactose can serve as the donor of galactose units in a number of synthetic pathways, including synthesis of lactose, glycoproteins, glycolipids, and glycosaminoglycans. If galactose is not provided by the diet, all tissue requirements for UDP-galactose can be met by the action of *UDP-hexose-4-epimerase* on UDP-glucose, which is efficiently produced from glucose-1-P.



ALDOSE REDUCTASE

- Enzyme is present in liver, lens, nerve tissue, seminal vesicles.
- Physiologically unimportant in galactose metabolism unless galactose levels are high (as in galactosemia).
- Elevated galactitol can cause cataract.

GALACTOKINASE DEFICIENCY

- Causes galactosemia and galactosuria.
- Causes galactitol accumulation if galactose is present in the diet

CLASSICAL GALACTOSEMIA

- *Uridyltransferase* deficiency.
- Causes galactosemia and galactosuria.
- Accumulation of galactose-1-P and galactitol in nerve tissue, lens, liver, and kidney, causing liver damage, severe mental retardation and cataract.
- Therapy: Rapid diagnosis and removal of galactose (therefore lactose) from the diet.

Figure: Galactose metabolism.

Glycogen Storage Diseases (GSDs):

There are a group of inherited disorders associated with glycogen metabolism, familial in incidence and characterized by deposition of normal or abnormal type and quantity of glycogen in the tissues. The overall frequency of all forms of glycogen storage disease is approximately 1 in 20,000 to 25,000 live births.

1. Von Gierke's disease:

The enzyme deficiency is *glucose-6-phosphatase*. Liver cells and cells of renal tubular epithelial cells are loaded with glycogen which is normal in structure but metabolically not available.

Clinical and biochemical features:

1. Since very little glucose is derived from the liver, children with this disease tend to develop "hypoglycaemia". Glucose-6-P can not be converted to glucose due to deficiency of the enzyme.
2. Fat is utilized as energy source which leads to lipaemia, acidaemia and ketosis.
3. Excess acetyl CoA is diverted for cholesterol synthesis resulting to increase in cholesterol level, which may produce Xanthomas.
4. Increased fatty acid can produce fatty acid infiltration of liver.
5. Persistent hypoglycaemia can have two effects:

a) Hypoglycaemia inhibits insulin secretion which in turn inhibits protein synthesis which causes stunted growth (dwarfism).

b) Hypoglycaemia stimulates secretion of catecholamines, which cause muscle glycogen to break down producing lactic acid and lactic acidosis.

6. Increased blood lactic acid competes with urate excretion by kidneys leading to increased blood uric acid level. There is also evidence that there is increase in uric acid synthesis in those children who develop symptoms of gout. Many of these children die young.

2. Pompe's disease:

Its fatal and is characterized by a deficiency of lysosomal α-1→4 and 1→6 glucosidase (*acid maltase*), whose function is to degrade glycogen, which otherwise accumulates in the lysosomes. The most tissues affected are heart, liver, smooth and striated muscles. Nearly all tissues contain excessive amount of normal glycogen.

Clinical and biochemical features are enlargement of heart (cardiomegaly), and muscle hypotonia leading to muscle weakness.

3. Forbe's disease (Limit dextrinosis):

The enzyme deficiency is *debranching enzyme*. Glycogen structure-limit dextrin type, abnormal short or missing outer chains. Organs involved are liver, heart, and muscles. Clinical and biochemical features are hepatomegaly, moderate hypoglycaemia, acidosis, progressive myopathy.

4. Andersen's disease (Amylopectinosis):

The enzyme deficiency is *branching enzyme*. Glycogen deposited is abnormal type, few branch points and very long inner and outer unbranched chains (looks similar to amylopectin). The affected organs are liver (mainly affected), heart, muscles, and kidneys.

Clinical and biochemical features are hepatomegaly, splenomegaly, ascites, moderate hypoglycaemia, nodular cirrhosis of liver and hepatic failure. This disease is usually fatal. Longest survival reported as 4 years.

5. Mc Ardle's disease:

The enzyme deficiency is *muscle phosphorylase*. Excess of glycogen (normal in structure) is deposited in skeletal muscles.

Clinical and biochemical features:

Muscle cramps on exercise, pain, weakness and stiffness of muscles. No lactate is formed. Muscles recover on rest, due to utilization of fatty acids for energy.

Epinephrin test:

After administration of epinephrin / or glucagon, rise in blood glucose occurs which shows that hepatic phosphorylase activity is normal.

6. Her's disease:

The enzyme deficiency is *liver phosphorylase*. Glycogen deposited is normal in structure. Liver is the main organ affected.

Clinical and biochemical features hepatomegaly, mild to moderate hypoglycaemia, mild acidosis.

METABOLISM OF LIPIDS

with Dr. Saeed

METABOLISM OF LIPIDS

19/4/09

Digestion & absorption of lipids:

An adult ingests about 60 to 150 gm of lipids per day, of which more than 90% is normally triacylglycerols (TG_s). The remainder of the dietary lipid is made up of cholesterol, cholesterol esters, phospholipids, and unesterified ("free") fatty acids.

The digestion of lipid is started in mouth by the action of the *lingual lipase* which is secreted by the dorsal surface of the tongue (Ebner's gland). The pH range of activity is 2 to 7.5 (optimal pH value is 4.5). *Lingual lipase* is more active on TG_s having shorter fatty acid (FA) chains. Milk fat appears to be the best substrate for this enzyme, because milk fats rich with short and medium FA chains. The released short chain FA_s can be absorbed directly from the stomach wall and enter the portal vein. Stomach secretes an enzyme called as *gastric lipase* which is active only at neutral pH and is therefore of little use in the adult stomach where the pH is low. However, in infants, whose stomach pH is nearer neutrality and whose diets often contain milk lipids, *gastric lipase* may play a role in lipid digestion. The gastric contents (acidic chyme) reaches the duodenum.

Cells in the mucosa of the jejunum and lower duodenum produce a small peptide hormone, **cholecystokinin** (CCK; also called **pancreozymin**), in response to the presence of lipid contents of the chyme entering these regions of the upper small intestine. This hormone acts on the gall bladder (causing it to contract and release bile) and on the exocrine cells of the pancreas (causing them to release digestive enzymes). It also decreases gastric motility, resulting in a slower release of the gastric contents into the small intestine. Other intestinal cells produce another small peptide hormone, **secretin**, in response to the low pH of the chyme entering the intestine. Secretin causes the pancreas to release a watery solution rich in bicarbonate that helps neutralize the pH of the intestinal contents, bringing it to the approximate pH for enzymic digestive activity.

In the duodenum the digestion of the dietary lipids possess a special problem because of: **a)** the insolubility of fats in water; and **b)** the lipolytic enzymes, like other enzymes, are soluble in an aqueous medium. This problem is solved by the emulsification of the dietary lipids by bile salts. Emulsification increases the surface area of the lipid droplets, so that the digestive enzymes can act effectively. Bile salts help in combination of *lipase* in the intestinal lumen with two molecules of a small protein, **colipase**, secreted by the pancreas. This combination enhances the *lipase* activity.

Pancreatic juice has been shown to contain a number of lipolytic enzymes: *pancreatic lipase* hydrolyzes the TG molecules which preferentially removes the fatty acids at carbon 1 and 3. The primary products of hydrolysis are a mixture of 2-monoacylglycerol (2-MG) and free fatty acids. *Cholesterol esterase* hydrolyzes cholesterol ester molecules whereas cholesterol and free fatty acids are

the end products. *Phospholipase A_2* removes the fatty acid at carbon 2 of the phospholipids, leaving a lysophospholipid.

Absorption of lipids:

Free fatty acids, free cholesterol, and 2-MG are the primary products of dietary lipid degradation in the jejunum. These, together with bile salts, form mixed micelle-clusters of amphipathic lipids that coalesce with their hydrophobic groups on the inside and their hydrophilic groups on the outside of the cluster, and which are soluble in the aqueous environment of the intestinal lumen. The mixed micelles approach the primary site of lipid absorption, the brush border membrane of the intestinal mucosal cells. The products of lipid digestion enter the intestinal mucosal cells by simple diffusion through the cell membrane. Inside the intestinal mucosal cells resynthesis of TG, cholesterol esters, and phospholipids is started by activation of the fatty acids to fatty acyl CoA by *fatty acyl CoA synthetase*. Using the fatty acyl CoA derivatives, the 2-monoacylglycerols absorbed into the intestinal mucosal cells are converted to TG_s by a family of *acyltransferases* that recognize fatty acyl CoA_s of

2-monoacylglycerol + 2 fatty acyl CoA ⟶ TG + 2 CoA

specific chain length. Short and medium chain-length fatty acids are not converted to their CoA derivatives, but rather are released into the portal circulation where they are carried by serum albumin to the liver. A family of *acyltransferases* is responsible for the reacylation of lysophospholipids and cholesterol, producing phospholipids and cholesterol esters. In the intestinal mucosal cells, the resynthesized TG_s, cholesterol esters, phospholipids, cholesterol and a specific protein, apolipoprotein B-48 (synthesized by intestinal mucosal cells. If this protein is not synthesized, TG_s accumulate in these producing the genetic disorder **congenital abetalipoproteinemia**) are bind together to form a lipoprotein complex called as **chylomicron**. Chylomicrons are released by exocytosis from intestinal mucosal cells into the lymphatic vessels and later enter blood through the thoracic duct. TG contained in chylomicrons is hydrolyzed to glycerol and free fatty acids by *lipoprotein lipase*. This enzyme is synthesized primarily by the adipocytes and skeletal muscle cells. The result of the deficiency of *lipoprotein lipase* is a massive **chylomicronemia (familial type I hyperlipoproteinemia)**. The free fatty acids derived from the hydrolysis of TG may directly enter adjacent muscle cells or adipocytes, where they are used in the synthesis of TG molecules, which are stored until the fatty acids are needed by the body. Glycerol that is released from TG is used almost exclusively by the liver to produce glycerol-3-P, which can enter glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate (DHAP). After the action of lipoprotein lipase, chylomicrons becomes a so called **chylomicron remnant** (which contain cholesterol esters, phospholipids, protein, and some TG) are

taken up by the liver, where they are hydrolyzed to their component parts. If removal of chylomicron remnants from the plasma is defective, they accumulate in the plasma. This is seen in **familial type III hyperlipoproteinemia**. The overall functions of the chylomicrons are thus to deliver exogenous fatty acids to muscle and adipose tissue and to carry dietary cholesterol to the liver.

β-Oxidation of fatty acids:

When the energy supply from the diet (glucose) becomes limited, the human responds to the deficiency with a hormonal signal that is transmitted to the adipose tissue by the release of epinephrine and other hormones. The hormone binds to its specific receptor on the plasma membrane of the adipocyte and stimulates the synthesis of cAMP as follows:

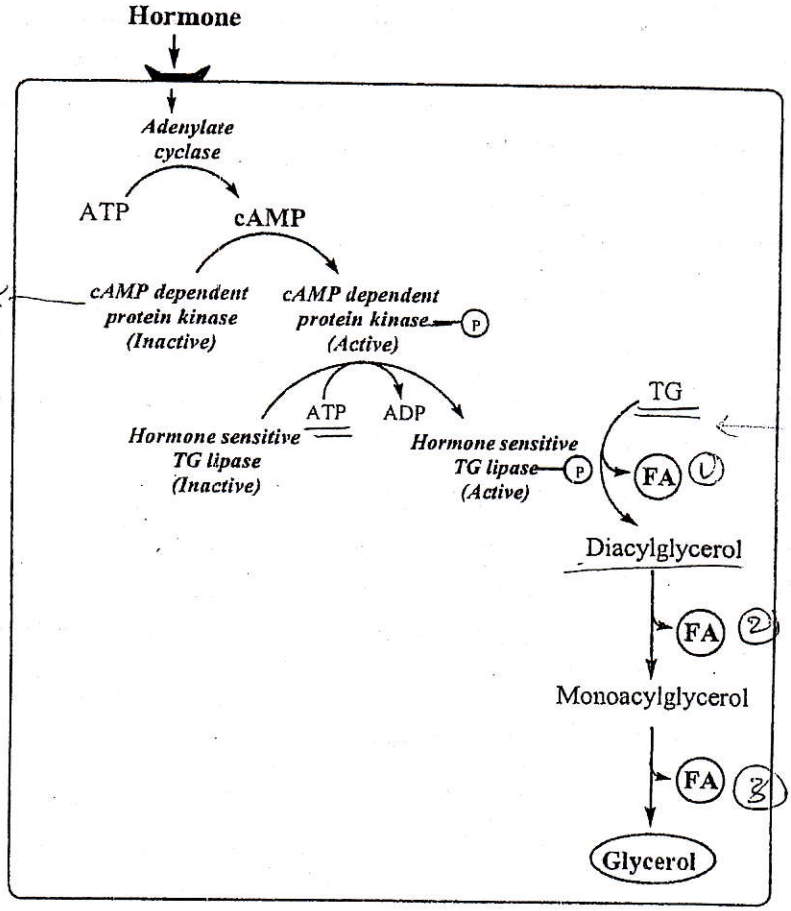


Figure : Influence of hormones on adipose tissue metabolism.

Glycerol and free fatty acids leave the adipocyte to the blood stream by passive diffusion. Glycerol is removed by the liver for glucose production (gluconeogenesis), or converted to dihydroxyacetone phosphate which enters glycolysis pathway. In the blood stream, the circulating free fatty acids are carried out by albumin where are taken up by various tissues for β-oxidation. After the fatty acid is taken up by the cell, it is activated to fatty acyl CoA by *fatty acyl CoA synthetase* in the

cytosol. Because β-oxidation occurs in the mitochondrial matrix, the fatty acid must be transported across the mitochondrial inner membrane, which is generally impermeable to bulky, polar molecules such as coenzyme A. Therefore, a specialized carrier (**carnitine**) in this membrane transports the acyl group from the cytosol into the mitochondrial matrix. First, an acyl group is transferred from the cytosolic coenzyme A to carnitine by *carnitine acyltransferase I*, forming acylcarnitine. The enzyme is located on the outer surface of the inner mitochondrial membrane. Second, the acyl carnitin group is transported across the membrane to the mitochondrial matrix, where it is transferred to another molecule of coenzyme A by *carnitine acyltransferase II* on the inner surface of the inner mitochondrial membrane.

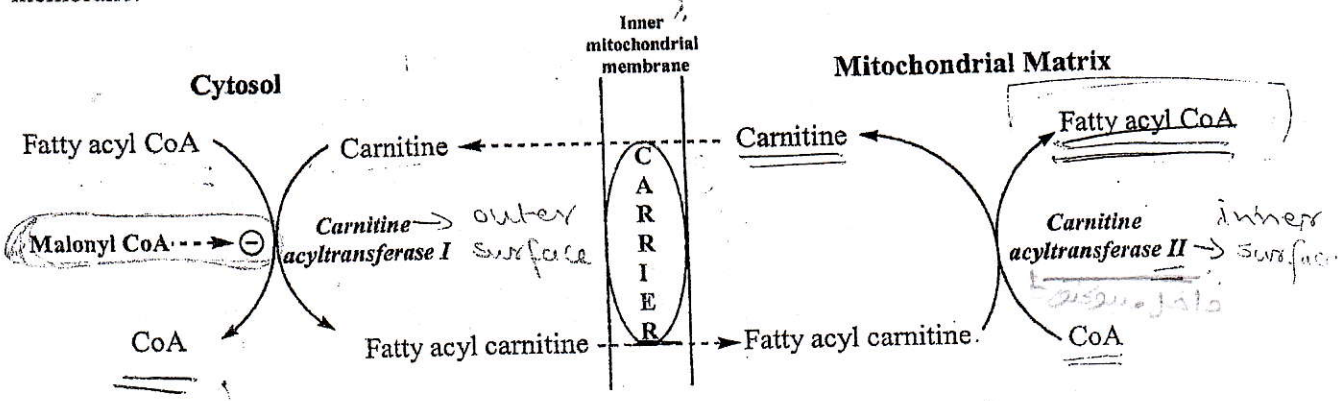


Figure : Carnitine shuttle.

Reactions of β-oxidation:

The first cycle of β-oxidation is shown in Figure . It consists of sequence of four reactions that result in the shortening of the fatty acid chain by two carbons. The steps include an oxidation that produces $FADH_2$, hydration, a second oxidation that produces NADH, and a thiolytic cleavage that releases a molecule of acetyl CoA. These four steps are repeated for saturated fatty acids of even-numbered carbon chains (n/2) – 1 times (where n is the number of carbons), each cycle producing an acetyl group plus one NADH and one $FADH_2$. The final thiolytic cleavage produces two acetyl groups.

* The important step in the β-oxidation is the acylation of the F-A.
* all enzymes of β-oxidation are found in the mitochondria.

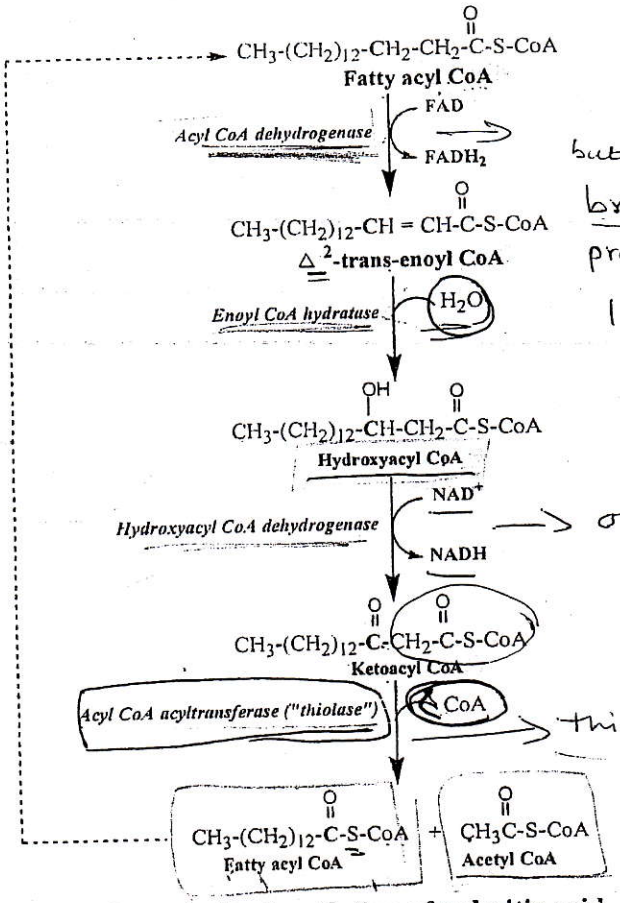


Figure : Steps in the β-oxidation of palmitic acid.

Energy yield from fatty acid oxidation:

The energy yield from the β-oxidation pathway is high. For example, the oxidation of a molecule of stearic acid (18 C) to CO_2 and H_2O yields the following number of ATP_s:

Chemical step	Happens	ATP yield
Activation (Stearic acid ⟶ Stearyl CoA)	Once	-2
Dehydrogenation (acyl CoA ⟶ transenoyl CoA), producing $FADH_2$	8 times	16
Dehydrogenation (hydroxyacyl CoA ⟶ ketoacyl CoA), producing NADH	8 times	24
Production of acetyl CoA	12 x 9 times	108
Total − 2 ATp = Result		146

* How many times the four steps repeat ?
equal = (FADH).

if it ask to calculate the ATP_5 from odd nu. , finally minuse 3 ATP 2 = activation , 1 = propanyle coA

Oxidation of fatty acids with an odd number of carbons:

The β-oxidation of a saturated fatty acid with an odd number of carbon atoms proceeds by the same reaction steps as that of fatty acids with an even number of carbon atoms, until the final three carbons are reached. This compound, **propionyl CoA**, is metabolized by a two-step pathway (Figure). First, propionyl CoA is carboxylated, forming methylmalonyl CoA by *propionyl CoA carboxylase*. Next, the carbons of methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the TCA cycle. The enzyme, *methylmalonyl CoA mutase*, requires a coenzyme form of vitamin B_{12} (deoxyadenosylcobalamin) for its action. In patients with vitamin B_{12} deficiency, both propionate and methylmalonate are excreted in the urine. Inherited absence of the *mutase* in children results in metabolic acidosis (**methylmalonic aciduria**) and develop mental retardation and finally death during childhood.

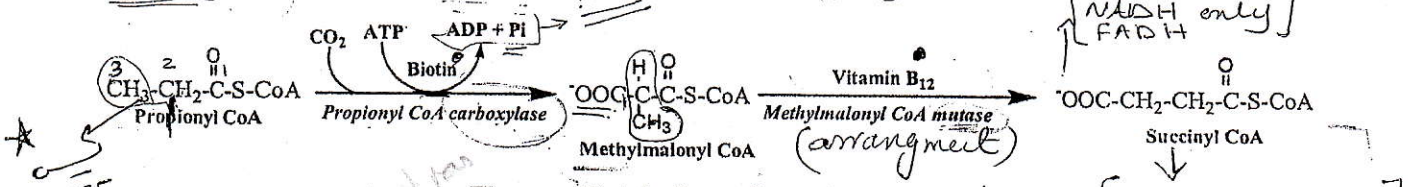


Figure : Metabolism of propionyl CoA

Diphtheria:

In diphtheria, bacteria produce a toxin that causes fatty acid accumulation and infiltration of myocardium (heart muscles). As a result, patients who have had diphtheria may have a weakened myocardium and be prone to heart failure. It is known that diphtheria toxin reduces the carnitine level in tissues. Carnitine is needed to transport fatty acids through the membrane to the inside of the mitochondria, where all the enzymes of β-oxidation are located. In the absence of carnitine, the fatty acids can not enter and so can not be metabolized. Thus, the diphtheria toxin's effect on carnitine causes the accumulation of fat in the heart tissues.

Carnitine deficiency:

Deficiency of carnitine can occur in:

a) New borns-specially premature infants-owing to inadequate synthesis or renal leakage.

b) Adults:

i) losses can occur in hemodialysis.

ii) in patients with organic acidurias carnitine is lost in urine being conjugated with organic acid.

Clinical features:

i) **Hypoglycemia**-due to reduced gluconeogenesis resulting from fatty acid oxidation.

ii) Impaired **ketogenesis** in the presence of raised plasma free fatty acids.

iii) Accumulation of lipids.

iv) Muscular weakness and myoglobinuria.

Treatment:

Oral therapy with carnitine.

De novo biosynthesis of fatty acids:

In humans, fatty acid synthesis occurs primarily in liver and lactating mammary glands and, to a lesser extent, in adipose tissue and kidney. The synthesis of fatty acids using acetyl CoA derived primarily from the excess intake of carbohydrates. The process incorporates carbons from acetyl CoA into the growing fatty acid chain, utilizing ATP and NADPH, and HCO_3^-. Fatty acid biosynthesis is a cytosolic process.

Acetyl CoA is synthesized in mitochondria and to be used in fatty acid biosynthesis must be transported out side to the cytosol. Because it can not cross the mitochondrial membrane, it is transported to the cytosol in the form of citrate produced by the condensation with oxaloacetate. Citrate is transported out by a transporter protein in exchange of malate. Once in cytosol an enzyme *citrate lyase* cleaves citrate with the help of ATP to form acetyl CoA and oxaloacetate.

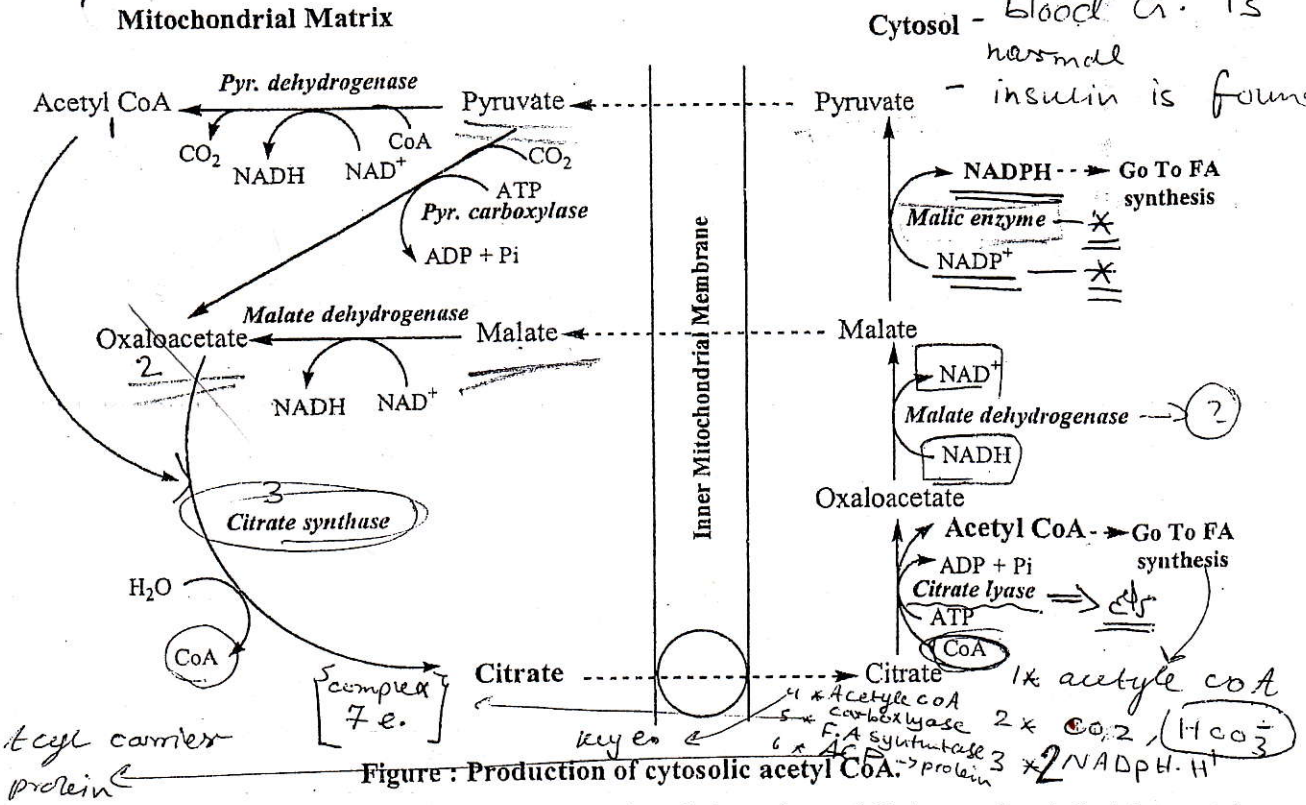


Figure : Production of cytosolic acetyl CoA.

Fatty acid synthesis is started by the carboxylation of acetyl CoA to malonyl CoA by ***acetyl CoA carboxylase*** (rate-limiting enzyme) with the help of ATP. The remaining series of reactions of fatty acid synthesis are catalyzed by ***fatty acid synthase*** (a multienzyme complex of seven enzymes plus a

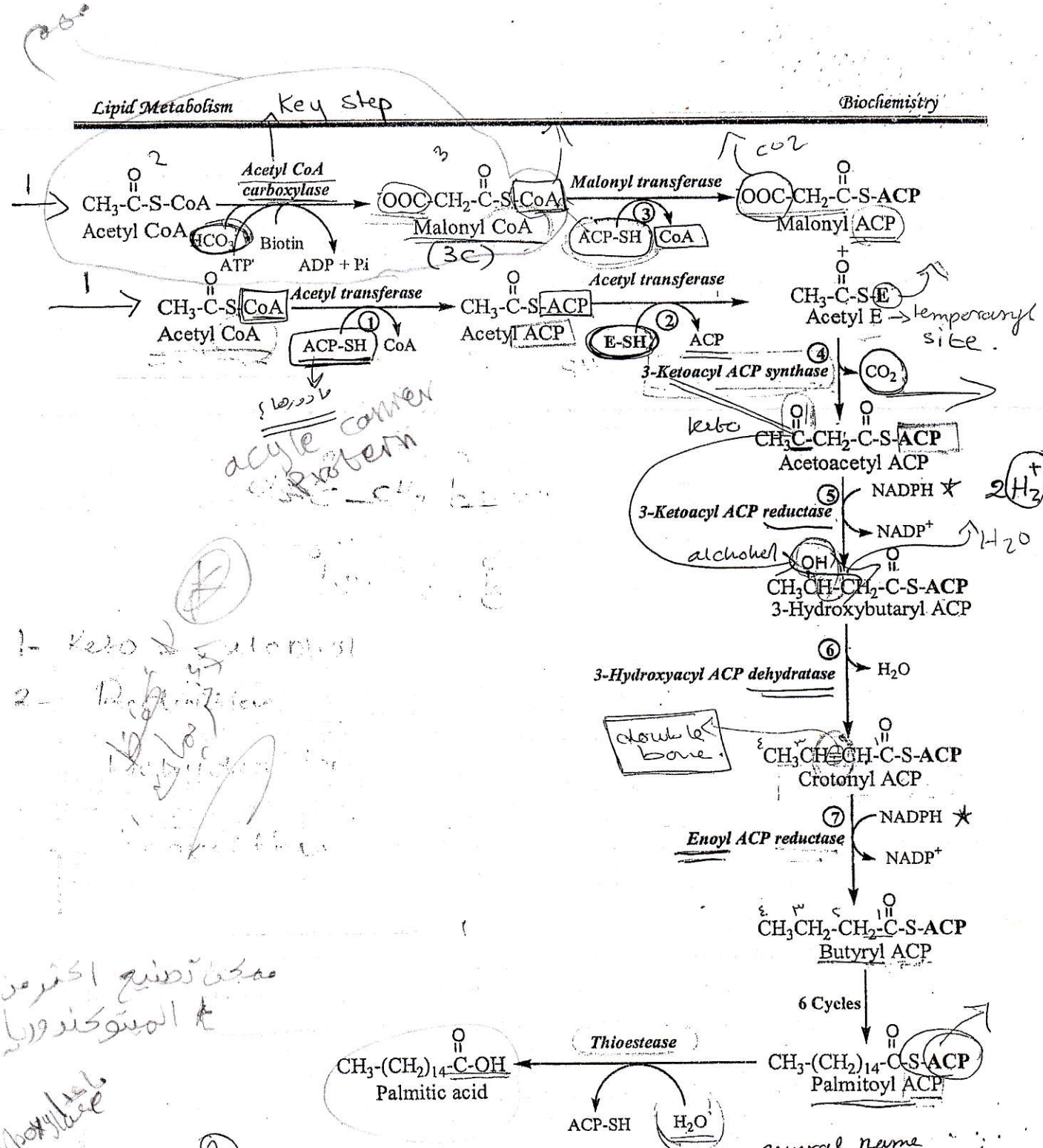


Figure ( ): Synthesis of palmitic acid.

protein called as acyl carrier protein "ACP"). ACP carries acetyl and acyl units on its terminal thiol (-SH) group during fatty acid synthesis.

(1)A molecule of acetate is transferred from acetyl CoA to the –SH group of the ACP. (2) Next, this two-carbon fragment is transferred to a cysteine residue on the enzyme, which acts as a temporary holding site. (3) The now-vacant ACP accepts a three-carbon malonate unit from malonyl

CoA. (4) The acetyl group attacks the malonyl group, which losses the CO_2 originally added by *acetyl CoA carboxylase.* The loss of free energy from this decarboxylation drives the reaction. (5) The keto group is reduced to an alcohol. (6) A molecule of water is removed to introduce a double bond. (7) A second reduction step occurs. The result of these seven steps is production of a four-carbon compound whose three terminal carbons are fully saturated, and that remains attached to the ACP. These seven steps are repeated, beginning with the transfer of the four-carbon chain from the ACP to the peripheral cystein side group, the attachment of a molecule of malonate to the ACP, and the condensation of the two molecules liberating CO_2. The carbonyl group at the β-carbon is then reduced. This cycle of reactions is repeated seven times, each time incorporating a two-carbon unit (derived from malonyl CoA) into the growing fatty acid chain. Once the fatty acid reaches a length of 16 carbons, the synthetic process is terminated, producing a fully saturated molecule of palmitate.

Regulation of fatty acid biosynthesis:

The carboxylation (by *acetyl CoA carboxylase*) is the regulated step in fatty acid synthesis. The inactive form of *acetyl CoA carboxylase* consists of a protomer made of four subunits. The enzyme undergoes activation by citrate, which causes the protomers to polymerize. The enzyme can be inactivated by **malonyl CoA** or **palmitoyl CoA**, which causes its depolymerization. Palmitoyl CoA also inhibits pentose phosphate pathway, since this pathway is a major supplier of the NADPH needed for fatty acid synthesis. In the presence of epinephrine, the enzyme is phosphorylated and thereby inactivated. In the presence of insulin, *acetyl CoA carboxylase* is dephosphorylated and thereby activated.

Prolonged consumption of high-carbohydrate or fat-free diets causes an increase in enzyme synthesis, thus increasing fatty acid synthesis. Conversely, a high-fat diet or fasting causes a reduction in fatty acid synthesis by decreasing the synthesis of *acetyl CoA carboxylase.*

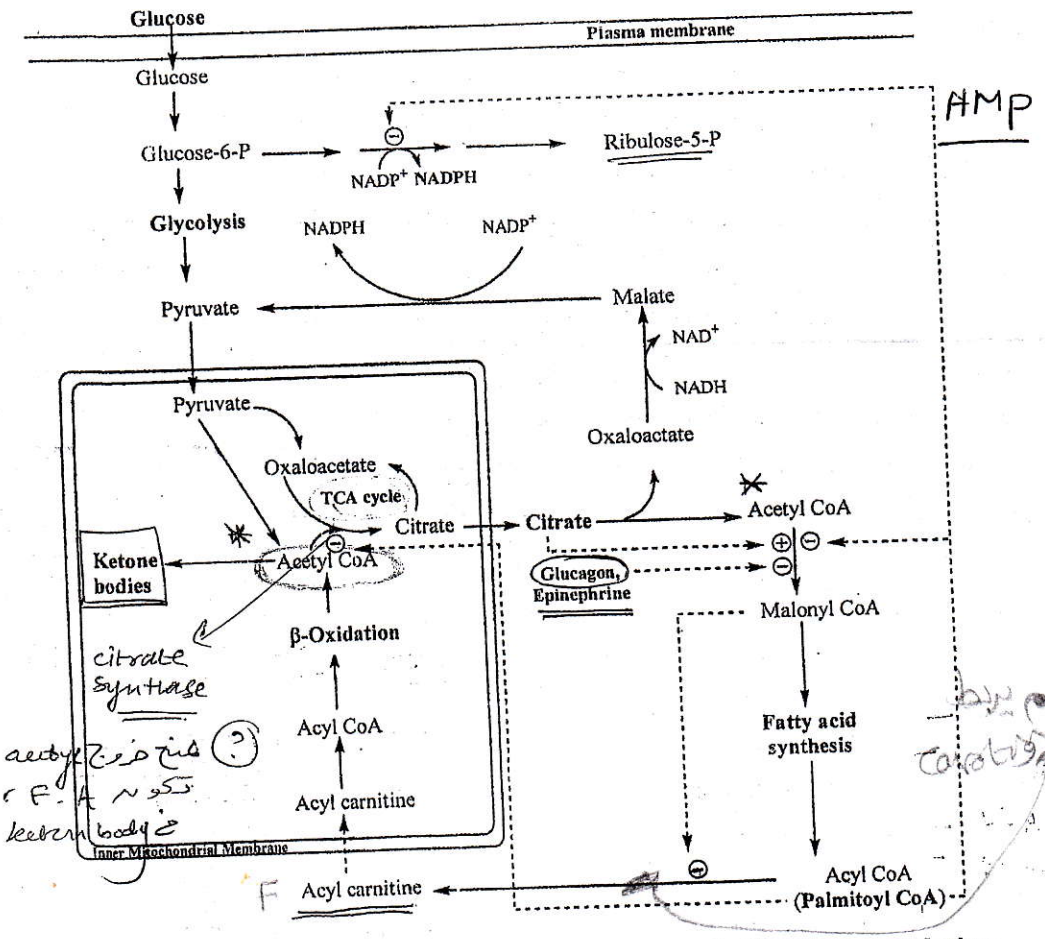


Figure : Overview of the conversion of carbohydrate to lipid and its regulation.

Elongation of fatty acids:

In mammalian systems, elongation of fatty acids can occur either in the endoplasmic reticulum or in mitochondria, and the processes are slightly different in these two loci. In the endoplasmic reticulum the sequence of reactions is similar to that which occurs in the cytoplasmic fatty acid synthesis in that the source of two-carbon units is again malonyl CoA, and NADPH provides the reducing power. The preferred substrate for elongation, by the *microsomal elongase* of chain elongation system in the smooth endoplasmic reticulum, in most cases is palmitoyl CoA.

The elongation system in mitochondria, is different from that in the endoplasmic reticulum in that the *mitochondrial elongase* uses acetyl CoA as the source of the added two-carbon units and both NADH and NADPH serve as reducing power. The process has little activity with acyl CoA substrates of 16 carbons or longer, suggesting that it serves primarily in the elongation of shorter-chain species.

Ketone bodies:

The normal synthesis of ketone bodies is known as **ketogenesis**. Liver (mitochondria) is the only organ which able to synthesize ketone bodies. Extrahepatic tissues can pick up ketone bodies from the blood and utilize them as an energy sources. Liver mitochondria have the capacity to divert any excess acetyl CoA derived from fatty acid or pyruvate oxidation into ketone bodies. **Acetoacetate**, ***β*-hydroxybutyrate**, and **acetone** , these three compounds are collectively known as ketone bodies.

Acetyl CoA is the starting material for ketogenesis. The liver is able to condense two acetyl CoA molecules to produce acetoacetyl CoA. A third molecule of acetyl CoA can combine with acetoacetyl CoA to produce *β*-hydroxy-*β*-methylglutaryl CoA (HMG CoA) catalyzed by ***HMG CoA synthase***. This enzyme is the **rate-limiting step** in the synthesis of ketone bodies and is present in significant quantities only in the liver. HMG CoA is cleaved by *HMG CoA lyase* to produce acetoacetate and acetyl CoA. Acetoacetate can be reduced to form *β*-hydroxybutyrate with NADH as a hydrogen donor, or it can be spontaneously decarboxylated to form acetone. Ketone bodies diffuse out the site of synthesis (liver) and taken up by extrahepatic tissues. Inside the mitochondria of these tissues,

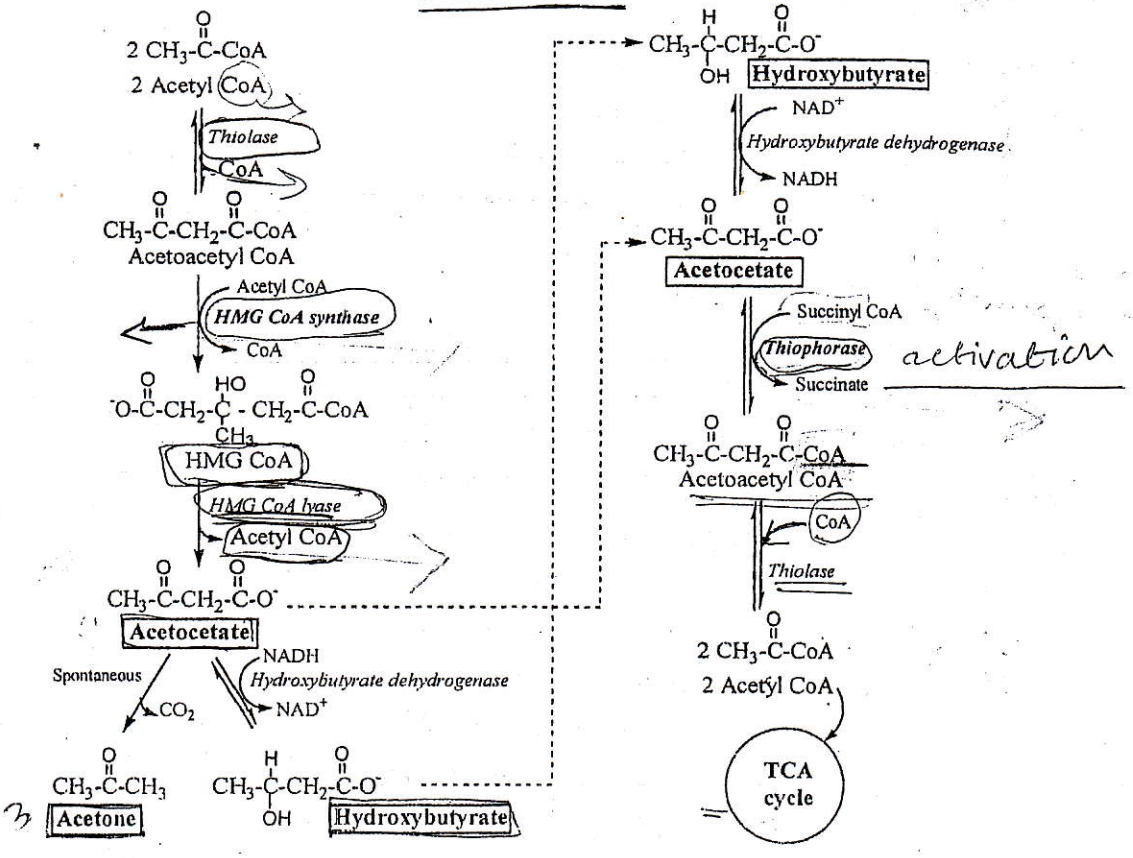


Figure : Formation and utilization of ketone bodies.

β-hydroxybutyrate can be converted back to acetoacetate by *β-hydroxybutyrate dehydrogenase*. Then acetoacetate receives a coenzyme A molecule from succinyl CoA and therefore activated to acetoacetyl CoA by ***succinyl CoA:acetoacetate CoA transferase*** (***thiophorase***). Liver lacks this enzyme, and therefore is unable to use ketone bodies as a fuel. Then acetoacetyl CoA is split to two molecules of acetyl CoA by *thiolase* and oxidized by the TCA cycle.

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (**ketonemia**) and eventually in the urine (**ketonuria**). These two conditions are seen most often in cases of starvation or severe diabetes mellitus. In diabetic individuals with severe ketosis, urinary excretion of ketone bodies may be as high as 5000 mg/day, and the blood concentration may reach 90 mg/dl (versus less than 3 mg/dl in normal individuals). An elevation of ketone body concentration in the blood results in **acidemia** [Each ketone body loses a proton (H^+) as it circulates in the blood, which lowers the pH of the body. Also, excretion of glucose and ketone bodies in the urine results in dehydration of the body. Therefore, the increased number of H^+, circulating in a decreased volume of plasma, can cause severe acidosis (**ketoacidosis**)].

Fate of acetyl CoA in liver:

Liver in both fed and fasting conditions, is capable of extracting 30% or more of free fatty acids (FFA_s) passing through it. So when FFA_s concentration in plasma is very high, substantial amount of FFA_s passes through the liver. Two fates await the FFA_s, taken up by the liver cells after activation to fatty acyl CoA. Either (**a**) they are esterified to form TG, phospholipids, and cholesterol esters, or (**b**) they undergo β-oxidation to form acetyl CoA. Acetyl CoA in turn is oxidized to CO_2 and water in TCA cycle. But if in excess, they are used for ketone body formation.

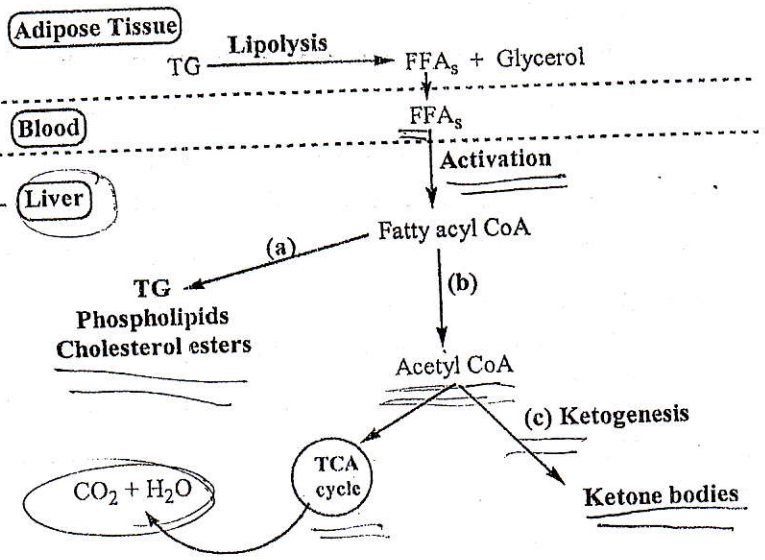


Figure : Fate of free fatty acids in liver.

In conditions of low glucose supply as in fasting or starvation, or when glucose can not be utilized, as in case of diabetes mellitus, the rate of TCA cycle slows down. Because, if there is no glucose, there will be no glycolysis, no pyruvate formation, and therefore no oxaloacetate production. Thus, even though the fatty acids are oxidized, not all the resulting acetyl CoA can enter the TCA cycle because there is not enough oxaloacetate. The fall in the concentration of oxaloacetate particularly within the mitochondria could cause impairment of TCA cycle to metabolize acetyl CoA. When demand for glucose are great as in diabetes mellitus, starvation, e.t.c., oxaloacetate is preferentially used up in formation of glucose from glucogenic amino acids, lactic acid, and glycerol (gluconeogenesis ↑), producing relative lack of oxaloacetate. The result is that acetyl CoA formed by oxidation of FFA_s can not be oxidized by TCA cycle and is diverted to formation of ketone bodies. Thus oxaloacetate can prevent ketosis by taking up acetyl CoA to form citrate, in its absence ketone bodies accumulate and may account for sever forms of **ketosis** (the production of ketone bodies in excess of the ability of the body to utilize them, therefore, they are excreted in urine and is called **ketonuria**).

The ketone bodies are not in themselves abnormal constituents of blood. Only when they are produced at a rate faster than the blood buffer can handle them are they a problem. The condition of excessive levels of ketone bodies in the blood is called **ketonemia**. As ketonemia becomes more and more advanced, the ketone bodies begin to appear in the urine, a condition called **ketonuria**. When there is a combination of ketonemia, ketonuria, and acetone breath, the overall state is called **ketosis**.

To leave the ketone body anions in the urine, the kidneys have to leave positive ions with them to keep everything electrically neutral. Na^+ ions, the most abundant cations, are used. One Na^+ ion has to leave with each acetoacetate ion, for example. The decrease in Na^+ ions draws out K^+ ions from the cells. This in turn impairs brain function and leads to coma. The solutes that are leaving the body in the urine can not, of course, be allowed to make the urine too concentrated. Otherwise, osmotic pressure balances are upset. Therefore increasing quantities of water must be excreted. To satisfy this need, the individual has a powerful thirst. Other wastes, such as urea, are also being produced at higher than normal rates, because amino acids are being sacrificed in gluconeogenesis. These wastes added to the demand for water to make urine. If, during a state of ketosis, insufficient water is drunk, then water is simply taken from extracellular fluids. The blood volume therefore tends to drop, and the blood becomes more concentrated. It also thickens and becomes more viscous, which makes the delivery of blood more difficult. Smaller quantities of nutrients reach the brain cells, and this too can causes coma. This, in addition to a combination of other developments, leads to coma and eventually death.

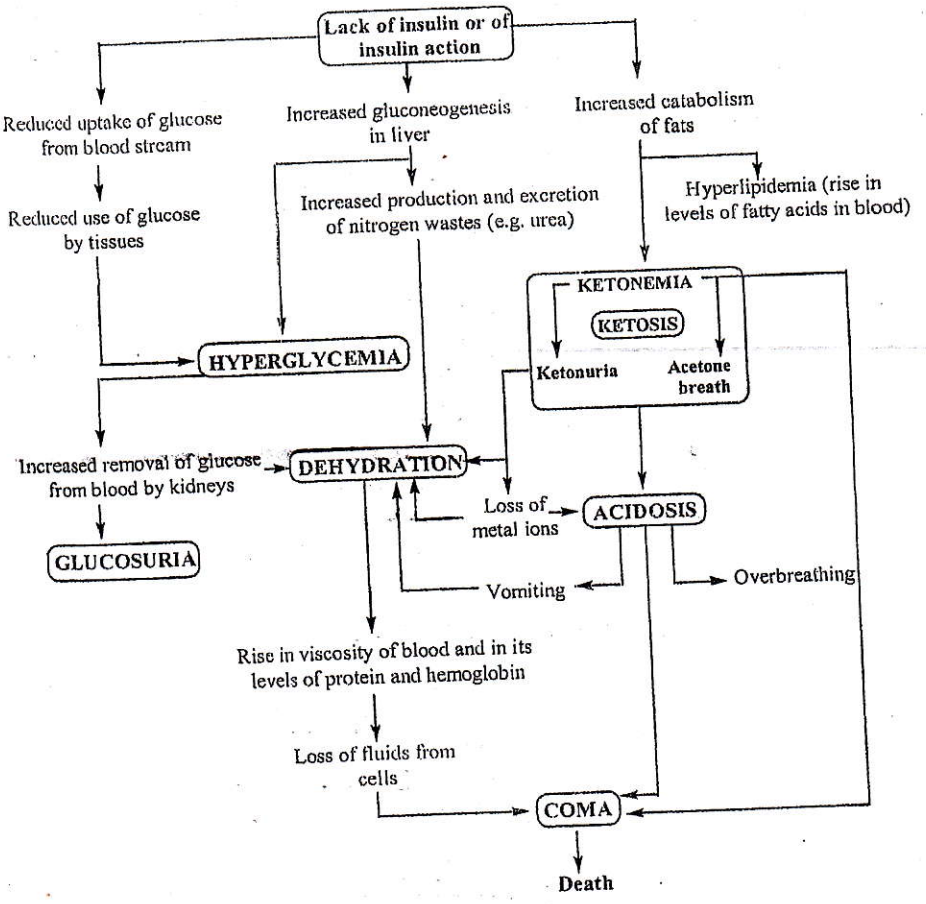


Figure : The principal sequence of events in untreated diabetes.

Metabolism of cholesterol:

Cholesterol is synthesized by virtually all tissues in humans. In mammals, about 80% to 95% of cholesterol synthesis takes place in cells of the liver and intestine. The remaining percentage of cholesterol synthesis is participated by adrenal cortex, reproductive tissues including ovaries, testes, and placenta. Cholesterol performs a number of essential functions in the body. For example, cholesterol is a component of all cell membranes and functions as a precursor of bile acids, steroid hormones, and vitamin D. The liver plays a central role in the regulation of the body's cholesterol balance. For example, cholesterol enters the liver's cholesterol pool from a number of sources, including dietary cholesterol, cholesterol synthesized by the extrahepatic tissues, and de novo synthesis of cholesterol by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, as a component of plasma lipoproteins sent to the peripheral tissues, or as bile salts secreted into the intestinal lumen.

Synthesis of cholesterol:

Synthesis of cholesterol is a cytosolic process. Cholesterol is the end product of a long multistep process started by a condensation of two molecules of acetyl CoA to form acetoacetyl CoA

which combines with another molecule of acetyl CoA to form HMG CoA. The HMG CoA is reduced to mevalonate, the reaction is catalyzed by ***HMG CoA reductase*** (it is the rate-limiting step in cholesterol synthesis). Mevalonate is next carried through a long series of reactions until cholesterol is made.

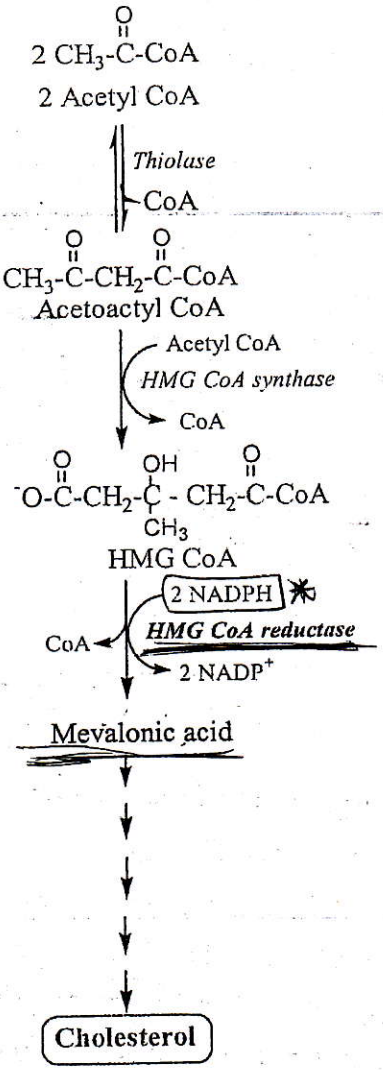


Figure : Synthesis of cholesterol.

Regulation of cholesterol synthesis:

The regulatory mechanisms must exist to balance the rate of cholesterol synthesis within the body against the rate of cholesterol excretion. An imbalance in this regulation can lead to an elevation in circulating levels of plasma cholesterol, causing the possibility of coronary artery disease, whereas excessive secretion of cholesterol into the bile can result in precipitation of cholesterol in the gall bladder and bile duct. *HMG CoA reductase* is the rate-limiting enzyme in cholesterol synthesis, and is subjected to different kinds of metabolic control.

1. Feedback inhibition: Cholesterol is a feedback inhibitor of *HMG CoA reductase*, thus decreasing further cholesterol synthesis.

Feeding of a cholesterol rich diet decreases cholesterol synthesis and vice versa. In contrast, feeding of diets high in carbohydrates tend to increase hepatic cholesterol synthesis. Fasting or starvation inhibits *HMG CoA reductase* and activates *HMG CoA lyase* to form ketone bodies. Increased intake of fats rich with saturated fatty acids increases serum cholesterol level, whereas substitution in the diet of saturated fatty acids by polyunsaturated fatty acids has beneficial effect and lowers serum cholesterol level. Increased fibers in the diet, caused an increased excretion of cholesterol and bile acids in feces and produced significant reduction in serum cholesterol.

2. Hormonal regulation: *HMG CoA reductase* activity is controlled hormonally through a complex cascade of enzyme activations and inhibitions similar to those described for the regulation of glycogen synthesis. The net effect is that glucagons favor formation of the inactive (phosphorylated) form of *HMG CoA reductase* and, hence, decreases the rate of cholesterol synthesis. In contrast, insulin favors formation of the active (unphosphorylated) form of *HMG CoA reductase* and results in an increase in the rate of cholesterol synthesis.

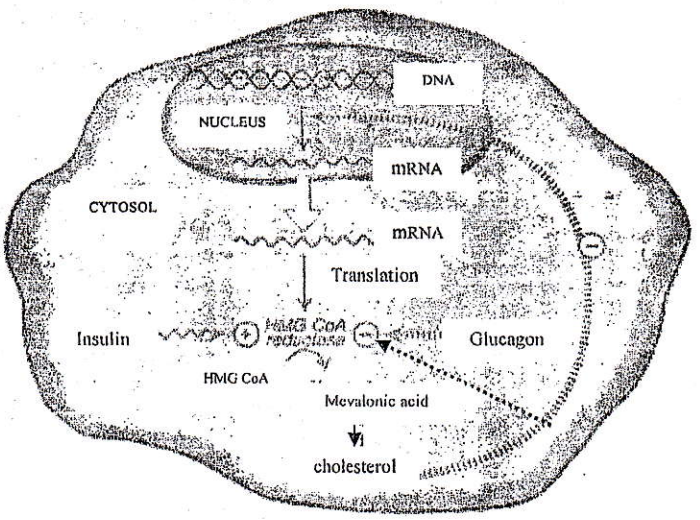


Figure : Regulation of HMG CoA reductase.

3. At the level of gene expression: The synthesis of cholesterol is also regulated by the amount of cholesterol taken up by the cells during lipoprotein metabolism. When the intake of cholesterol increased it causes a decrease in transcription of the HMG CoA reductase gene (the amount of mRNA

for *HMG CoA reductase* synthesis is reduced and therefore a decrease in the amount of the enzyme), leading to a decrease in de novo cholesterol synthesis.

4. Inhibition by drugs: Lovastatin and mevastatin are reversible , competitive inhibitors of *HMG CoA reductase*. They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia.

PLASMA LIPOPROTEINS:

The plasma lipoproteins are molecular complexes of lipids and specific proteins called apolipoproteins. These dynamic particles are in constant state of synthesis, degradation, and removal from the plasma. The lipoprotein particles include: the chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Lipoproteins function both to keep lipids soluble as they transport them in the plasma, and to provide an efficient mechanism for delivering their lipid contents to the tissues.

Composition of plasma lipoproteins:

The principal lipids carried by lipoprotein particles are triacylglycerols (TG) and cholesterol (free or esterified), obtained either from the diet or de novo synthesis. Lipoproteins are composed of a neutral lipid core (containing triacylglycerol or cholesteryl esters or both) surrounded by a shell of apolipoproteins (apoproteins), phospholipid, and nonesterified cholesterol all oriented so that their polar portions are exposed on the surface of the lipoprotein, thus making the particle soluble in aqueous solution.

1. **Size and density of lipoprotein particles:** The chylomicrons are the lipoprotein particles lowest in density and largest in size, and contain the most lipid and the smallest percentage of protein. VLDLs and LDLs are successively more dense, having a higher content of protein and a lower content of lipid. HDL particles are the most dense of the plasma lipoproteins. Plasma lipoproteins can be separated on the basis of their electrophoretic mobility, as shown in **Figure** ()

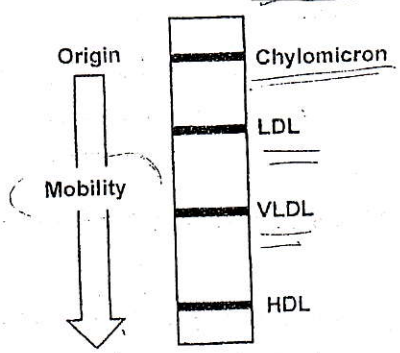


Figure ( ): Electrophoretic mobility of plasma lipoproteins.

2. **Apolipoproteins:** The apolipoproteins associated with lipoprotein particles have a number of diverse functions, including serving as structural components of the particles, providing

recognition sites for cell-surface receptors, and serving as activators or coenzymes for enzymes involved in lipoprotein metabolism. Apolipoproteins are divided by structure and function into classes A to H, with most classes having subclasses, for example, apoA-I and apoC-II.

Metabolism of chylomicron:

Chylomicrons are assembled in intestinal mucosal cells and carry dietary triacylglycerol, cholesterol, and cholesteryl esters (plus additional lipids made in these cells) to the peripheral tissues.

1. **Assembly of chylomicrons:** Apolipoprotein synthesis begins on the rough endoplasmic reticulum (RER); apolipoproteins glycosylated as they move through the ER and Golgi. The enzymes involved in triacylglycerol, cholesterol, and phospholipid synthesis are located in the smooth ER. Assemply of the apolipoproteins and the lipids into chylomicrons occurs during transition from the ER to Golgi, where they are packaged in secretory vesicles that are exported from the cell into the lymphatic system by exocytosis.
2. **Modification of nascent chylomicron particles:** The particle released by the intestinal mucosal cell is called a "nascent" chylomicron and contains apolipoprotein B-48 (apoB-48). When it reaches the plasma, the nascent chylomicron is rapidly modified, receving apo E (which, in conjunction with apoB-48, is recognized by hepatic receptors) and C apolipoproteins (including apoC-II, necessary for the activation of *lipoprptein lipase*. The source of these apolipoproteins is circulating HDL.
3. **Degradation of triacylglycerol by *lipoprotein lipase*:** *Lipoprotein lipase* is an extracellular enzyme that resides on the capillary walls of most tissues but is found predominantly in the capillaries of the adipose tissue and cardiac and skeletal muscle. *Lipoprotein lipase*, activated by apoC-II on circulating lipoprotein particles, hydrolyzes the triacylglycerol contained in these particles to yield fatty acids, and glycerol. [Note: Patients with a deficiency of *lipoprotein lipase* or apoC-II show a dramatic accumulation of triacylglycerol-rich lipoproteins in the plasma, for example, **type I hyperlipidemia (familial hyperchylomicronemia).**]
4. **Formation of chylomicron remnants:** As the chylomicron circulates and the triacylglycerol in its core is degraded by *lipoprotein lipase*, the particle decreases in size and increases in density. In addition, the C apolipoproteins are returned to the HDLs. The remaining particle is called a "remnant". In humans, these chylomicron remnants are removed from the circulation by the liver. Hepatocyte membranes contain lipoprotein recptors that recognize the combination of apolipoproteins B-48 and E. Chylomicron remnants bind to these receptors and are taken into the cells by endocytosis. The endocytosed vesicle then fuses with the lysosome, and the apolipoproteins, cholesteryl esters, and other components of the remnant are hydrolytically

degraded, releasing amino acids, free cholesterol, and fatty acids. The cholesterol released from the chylomicron regulates the rate of the de novo cholesterol synthesis in the liver by causing a decrease in cell content of *HMG CoA reductase* as well as by allosterically inhibiting the enzyme.

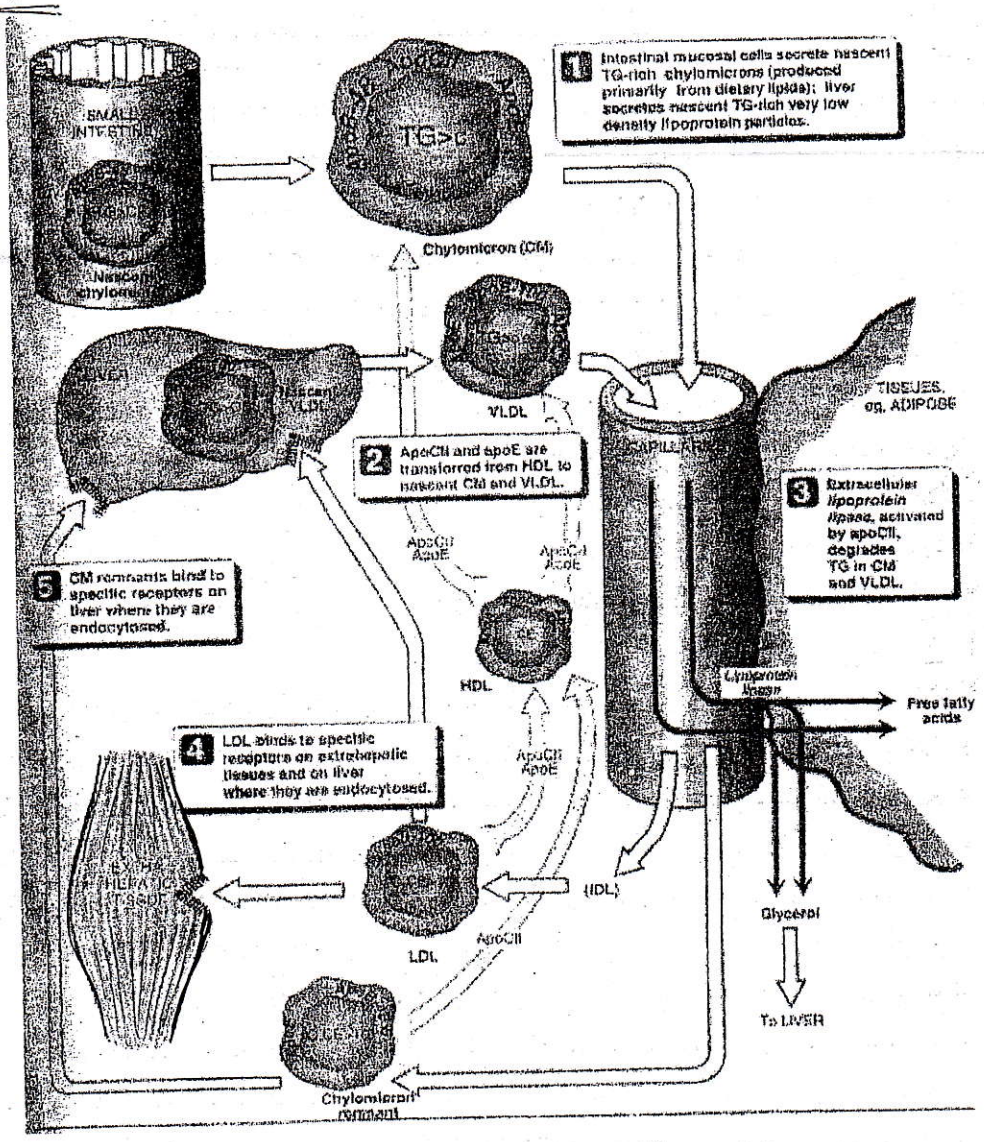


Figure ( ): Metabolism of plasma lipoproteins.

Metabolism of very low density lipoproteins (VLDL):

VLDLs are produced in the liver. They are composed predominantly of triacylgycerol, and their function is to carry this lipid from the liver to the peripheral tissues. There, the triacylglycerol is degraded by *lipoprotein lipase*. [Note: "**Fatty liver**" occurs in conditions in which there is an imbalance between hepatic triacylglycerol synthesis and the secretion of VLDLs].

1. **Release of VLDL:** VLDLs are released from the liver as nascent VLDL particles containing apolipoproteins B-100 and A-I. They must obtain apoC-II and apoE from circulatining HDL.
2. **Modification of circulating VLDL:** As VLDLs pass through the circulation their structure is altered. Triacylglycerol is removed by *lipoprotein lipase*, causing the VLDL to decrease in size and become more dense. Surface components, including the C and E apolipoproteins, are transferred to HDL. Finally, cholesteryl esters are transferred from HDL to VLDL in an exchange reaction that concomitantly transfers triacylglycerol or phospholipid from VLDL to HDL. This exchange is accomplished by **cholesterol ester transfer protein.**
3. **Production of LDL from VLDL in plasma:** After these modifications, the VLDL has been converted in the plasma to LDL. [Note: An intermediate-sized particle, the **intermediate density lipoprotein (IDL)** is observed durind the transition from VLDL to LDL in the plasma. IDL can also be taken up by cells through receptor-mediated endocytosis].

Clinical aspect: ApoB-48 and apoB-100 are most essential for chylomicrons and VLDL formation. In "abetalipoproteinemia"-a rare disease, apoB is not synthesized and hence chylomicrons and VLDL cannot form and lipid accumulate in intestinal mucosal cells and hepatic cells (**"fatty infilteration"**).

Metabolism of low density lipoproteins (LDL):

LDL particles retain apoB-100, but lose their other apolipoproteins to HDL. They contain much less triacylglycerol than their VLDL predecessors, and have a high concentration of cholesterol and cholesteryl esters.

1. **Receptor-mediated endocytosis:** The primary function of LDL particles is to provide cholesterol to the peripheral tissues. They do so both by depositing free cholesterol on the membranes of cells as they come in contact with the cell surface, and by binding to receptors on cell-surface membranes that recognize apolipoprotein B-100. A summary of the uptake and degradation of LDL particles is presented in **Figure ( )**. A similar mechanism is used for the cellular uptake and degradation of chylomicron remnants and HDLs by the liver.

[1]. LDL receptors are negatively charged glycoprotein molecules that are clustered in pits on the cell membranes. The intracellular side of the pit is coated with the protein **clathrin** which stabilizes the shape of the pit. [Note: A deficiency of functional LDL receptors causes a significant elevation in plasma LDL, and therefore of plasma cholesterol, but plasma triacylglycerol levels remain normal, for example, as in **type II hyperlipidemia (familial hyperbetalipoproteinemia)**. This can accelerate the progress of atherosclerosis]. تصلب الشرايين

[2]. After binding, the LDL are internalized as intact particles by endocytosis.

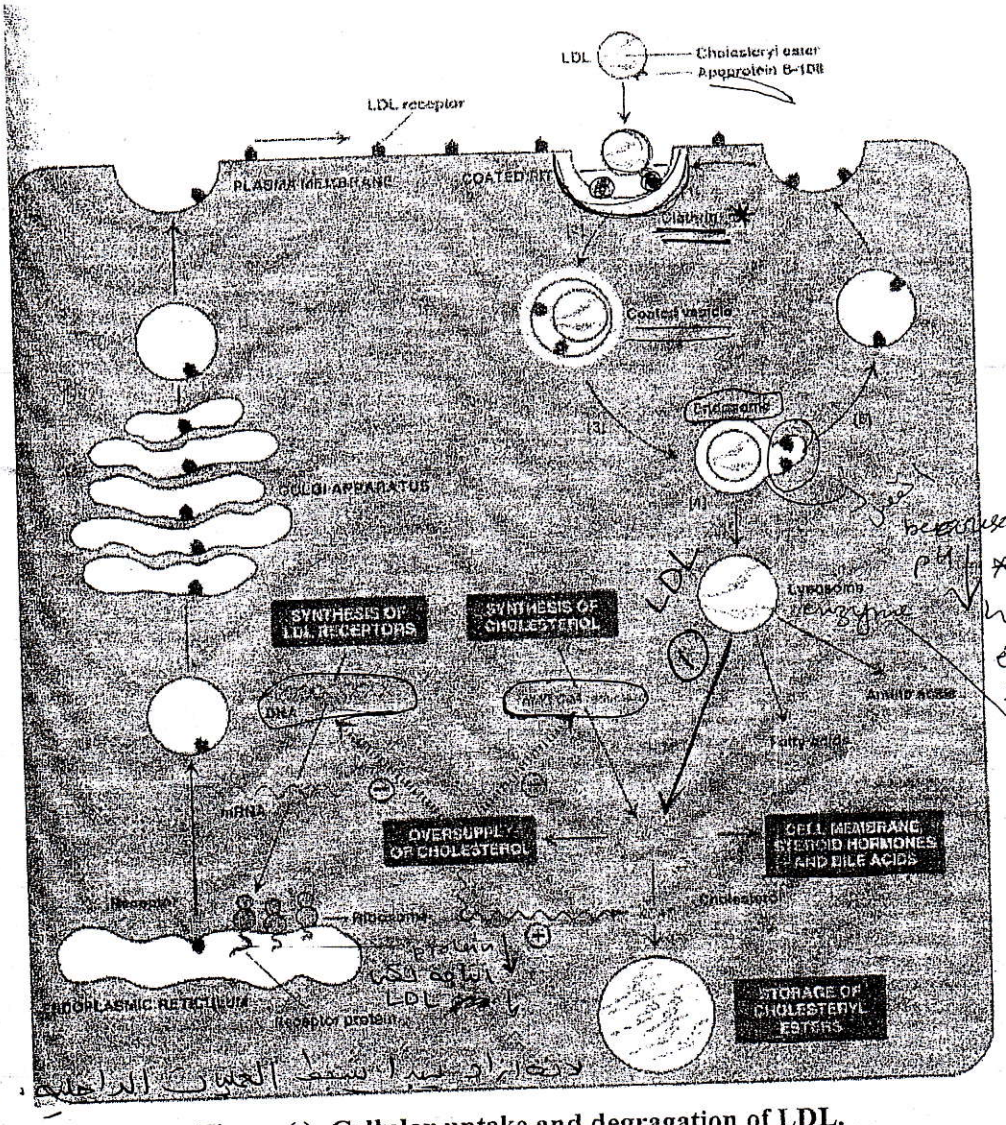


Figure ( ): Cellular uptake and degragation of LDL.

[3]. The vesicle containing the LDL rapidly loses its clathrin coat and fuses with other similar vesicles, forming larger vesicles called **endosomes**.

[4]. The pH of the contents of the endosome falls (due to the proton-pumping activity of *endosomal ATP$_{ase}$*), allowing separation of the LDL from the receptor. The receptors then migrate to one side of the endosome, whereas the LDLs stay free within the lumen of the vesicle.

[5]. The receptors can be recycled, whereas the lipoprotein remnants in the vesicle are degraded by lysosomal (hydrolytic) enzymes, releasing cholesterol, amino acids, fatty acids, and phospholipids. These compounds can be recycled by the cell.

2. **Effect of endocytosed cholesterol on cell cholesterol content:** The chylomicron remnant-, HDL-, and LDL-derived cholesterol affects cellular cholesterol content in several ways (see

Figure). **First,** *HMG CoA reductase* activity is inhibited by cholesterol so that de novo cholesterol synthesis decreases. **Second,** if the cholesterol is not required immediately for some structural or synthetic purpose, it is esterified by *acyl CoA cholesterol acetyltransferase* (*ACAT*). *ACAT* transfers a fatty acid from a fatty acyl CoA derivative to cholesterol, producing a cholesteryl ester that can be stored in the cell. The activity of this enzyme is enhanced in the presence of increased intracellular cholesterol. **Third,** synthesis of new LDL receptor protein is lowered by decreasing transcription on the LDL gene, so that further entry of LDL cholesterol into the cell is limited.

3. **Uptake of chemically modified LDL by macrophage scavenger:** In addition to the high specific receptor-mediated pathway for LDL uptake described above, circulating macrophages possess high levels of scavenger receptor activity. These receptors, which have broad ligand-

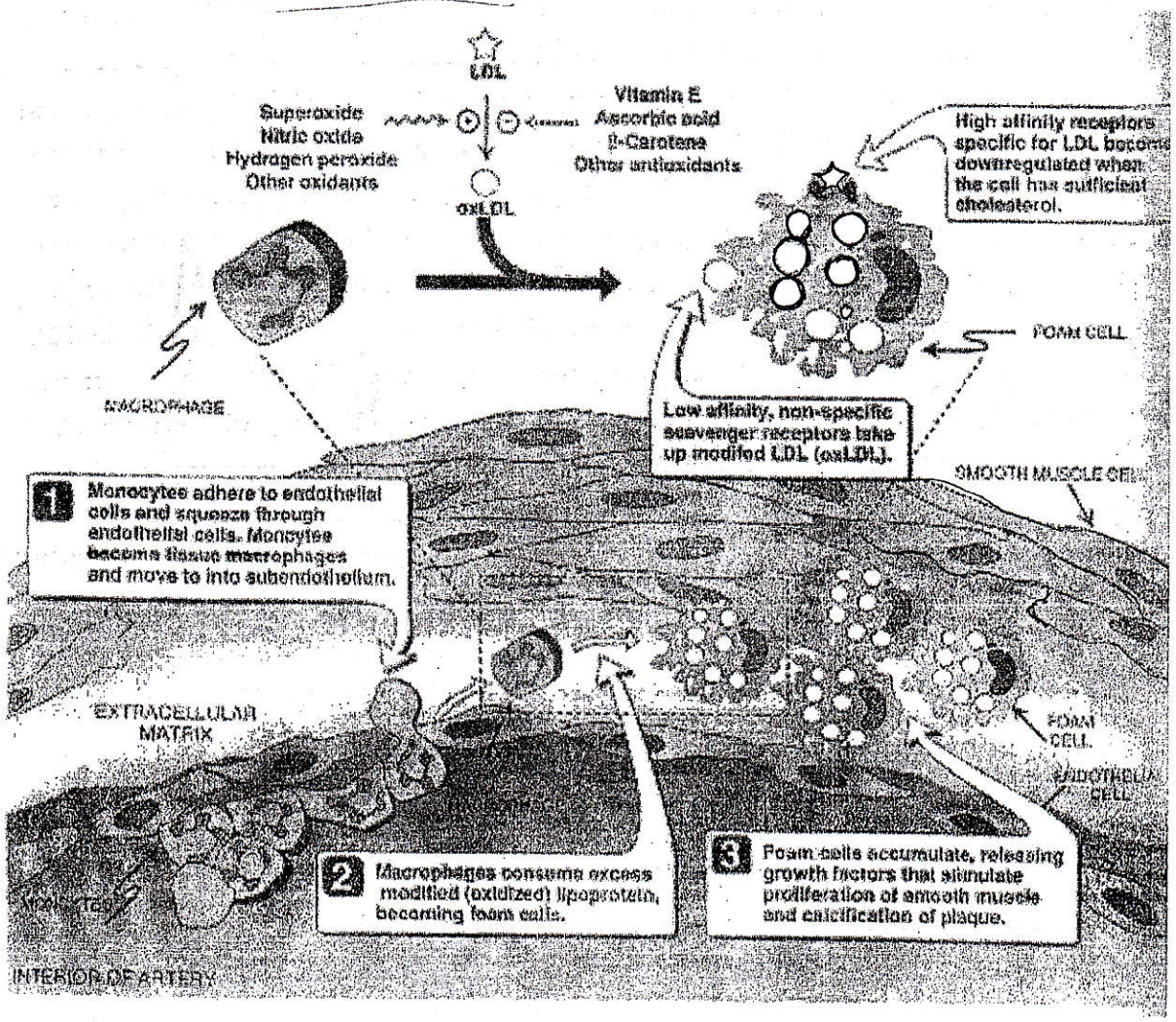


Figure ( ): Role of oxidized lipoproteins in plaque formation in arterial wall.

binding specificity, can mediate the endocytosis of chemically mediated LDL. Chemical modifications that convert circulating LDL into ligands that can be recognized by the receptors include acetylation or oxidation of apolipoprotein B. [Note: The initiating step in the modification of apo B involves the peroxidation of polyunsaturated fatty acids in the LDL lipids. This process can be inhibited by antioxidants such as vitamin E]. Unlike that taken up by the LDL receptors, the modified LDL taken up by macrophages does not regulate intracellular cholesterol levels, therefore cholesterol accumulates in these cells. Excessive uptake of modified LDLs by macrophages causes the transformation of these cells into "foam" cells, which, participate in the formation of atherosclerotic plaques.

Metabolism of high density lipoproteins (HDL):

HDL particles are synthesized in the liver and also in intestinal mucosal cells, and are released into the bloodstream by exocytosis. Nascent intestinal HDL contains only apoA, when it circulates, it acquires apoC and apoE. ApoC and apoE are only synthesized in liver and not in intestinal mucosal cells. Newly secreted hepatic HDL are disc-shaped particles containing predominantly unesterified cholesterol, a bilayer of phospholipid (largely lecithin), and a number of apolipoproteins including apoE, apoA, and apoC. They are rapidly converted to spherical particles as they accumulate cholesterol.[Note: HDL particles are excellent acceptors of unesterified cholesterol from the surface of cell membranes and from other circulating lipoproteins]. Once free cholesterol is taken up by the HDL, it is immediately esterified by *Lecithin-cholesterol acyltransferase* (*LCAT*), a plasma enzyme synthesized by the liver, which is activated by apoA-I of HDL itself. The fatty acid from carbon 2 of lecithin is transferred directly to the cholesterol, leaving lysolecithin. The resulting nonpolar cholesteryl esters move into the hydrophobic interior of the bilayer, whereas lysolecithin molecules are released from HDL to plasma, where they bind to albumin. These reactions, gradually changes the "discoid" HDL into "spherical" HDL. About two third of the cholesterol in the plasma is esterified with fatty acid. [Note: In liver disease, a decreased concentration of plasma cholesterol esters is observed. This may be due to either a deficiency in lecithin production or a lack of *LCAT*]. With the HDL-bound *LCAT*, the latter esterifies cholesterol into cholesteryl esters in HDL and thus maintains a "low concentration of free cholesterol" in HDL-particles, enabling the transfer of more cholesterol into the latter.

Fate of HDLs: Spherical HDL particles are taken up by the liver by receptor-mediated endocytosis, and the cholesteryl esters are degraded. The cholesterol thus released can be either repackaged in lipoproteins, converted into bile acids, or secreted into the bile for removal from the body.

HDL performs a number of important functions, including serving as a circulating reservoir of lipoproteins required for the proper metabolism of the other plasma lipoproteins (VLDL and chylomicrons); removing free (unesterified) cholesterol from extrahepatic tissues and esterifing it by using *LCAT*; transfering cholesteryl esters to chylomicron, VLDL and LDL (these lipoprotein may then transport those cholesteryl esters to liver) in exchange for triacylglycerol by means of the cholesteryl ester transfer protein (apoD), which is another protein component of HDL; carring cholesteryl esters to the liver, where the HDL is degraded and cholesterol released.

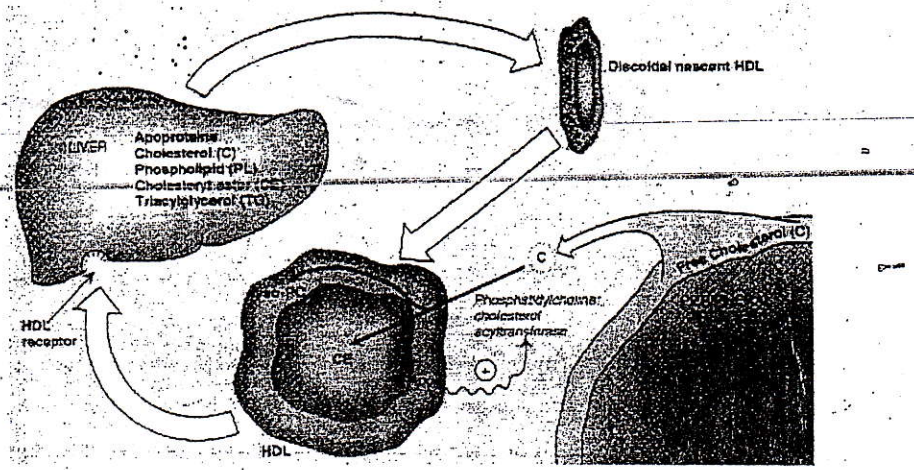


Figure ( ): Metabolism of HDL.

Scavenging action of HDL: HDL plays a major role in the removal of cholesterol from peripheral extrahepatic tissues and transport of this cholesterol to the liver where it is further metabolized. This has been called as "scavenging action" of HDL. HDL concentrations are inversely related to the insidence of coronary atherosclerosis, possibily because they reflect the efficiency of cholesterol-scavenging from the tissues "reverse cholesterol transport".

Clinical aspect: The lipoprotein particles, similar to "nascent" discoid HDL are found in plasma of patients with *LCAT* deficiency and also in plasma of patients with "obstructive jaundice".

METABOLISM OF AMINO ACIDS & PROTEINS

METABOLISM OF AMINO ACIDS & PROTEINS

Digestion & Absorption of Dietary Proteins:

A) Digestion by gastric secretion:

The digestion of proteins begins in the stomach, which secretes gastric juice, a unique solution containing hydrochloric acid and the proenzyme pepsinogen:

1. Hydrochloric acid: It is secreted by the parietal cells of the stomach. Hydrochloric acid functions to kill most bacteria (entering the gastrointestinal tract) and to denature proteins, making them more susceptible to subsequent hydrolysis by proteases.

2. Pepsin: The acid-stable endopeptidase is secreted by the serous cells of the stomach as inactive zymogen, pepsinogen. Pepsinogen is activated to pepsin either by HCl, or autocatalytically by other pepsin molecules that have already been activated. Pepsin releases peptides and a few free amino acids from dietary proteins.

B) Digestion by pancreatic enzymes:

On entering the small intestine, large polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases (trypsin, chymotrypsin, elastase, carboxypeptidases A & B) which are released as zymogens. The release and activation of the pancreatic zymogens is mediated by the secretion of the two polypeptide hormones of the digestive tract, cholecstokinin and secretin.

1. Activation of zymogens: ***Enteropeptidase*** (formerly called *enterokinase*), an enzyme synthesized by and present on the luminal surface of intestinal mucosal cells of the brush border membrane, converts the pancreatic zymogen trypsinogen to ***trypsin*** by removal of a hexapeptide from the NH_2-terminus of trypsinogen. Trypsin subsequently converts other trypsinogen molecules to trypsin. Enteropeptidase thus unleashes a cascade of proteolytic activity, because trypsin is the common activator of all the pancreatic zymogens (Figure). Each of these enzymes has a different specificity for the amino acid R groups adjacent to the susceptible peptide bond. For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine.

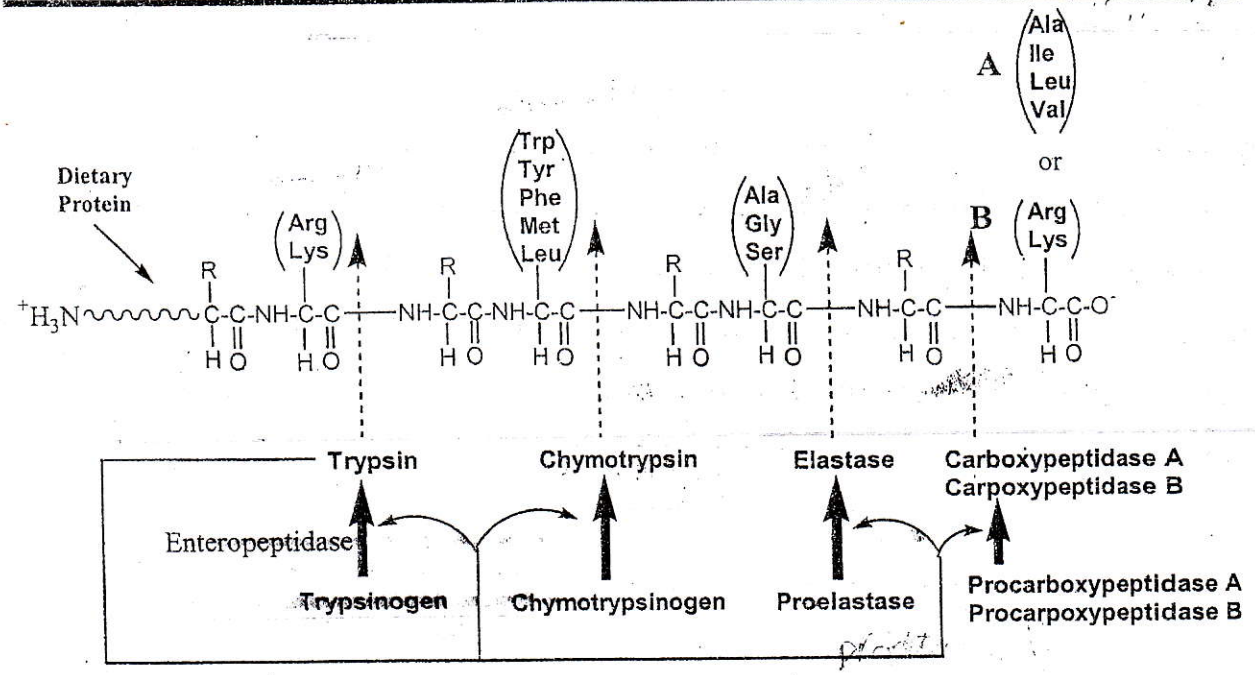


Figure : Cleavage of dietary proteins by proteases from the pancreas.

2. Abnormalities in protein digestion: In individuals with a deficiency in pancreatic secretion (for example, due to chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas), the digestion of fat and protein is incomplete. This results in the abnormal appearance of lipids, called **"steatorrhea"**, and undigested protein in the feces.

C) Digestion by enzymes of the small intestine: The luminal surface of the intestine contains ***aminopeptidase***, an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce free amino acids and smaller peptides. ***Tripeptidase*** acts on a tripeptide and produce a dipeptide and free amino acid. ***Dipeptidase*** hydrolyzes a dipeptide to produce two molecules of amino acids.

D) Absorption of amino acids & peptides:

Free amino acids and dipeptides are absorbed by the intestinal epithelial cells in which the dipeptides are hydrolyzed to amino acids in the cytosol before they enter the portal system. Thus, only free amino acids are found in the portal vein after a meal containing protein. These amino acids are either metabolized by the liver or released into the general circulation.

Amino acid pool:

Amino acids released by hydrolysis of dietary or tissue protein mix with other free amino acids distributed throughout the body, and collectively constitute the amino acid pool. The amino acid pool, containing about 100 gm of amino acids. About 75% of the amino acids obtained through hydrolysis of body protein are recaptured through the biosynthesis of new tissue protein. The remainder serve as precursors for the compounds shown in Figure .

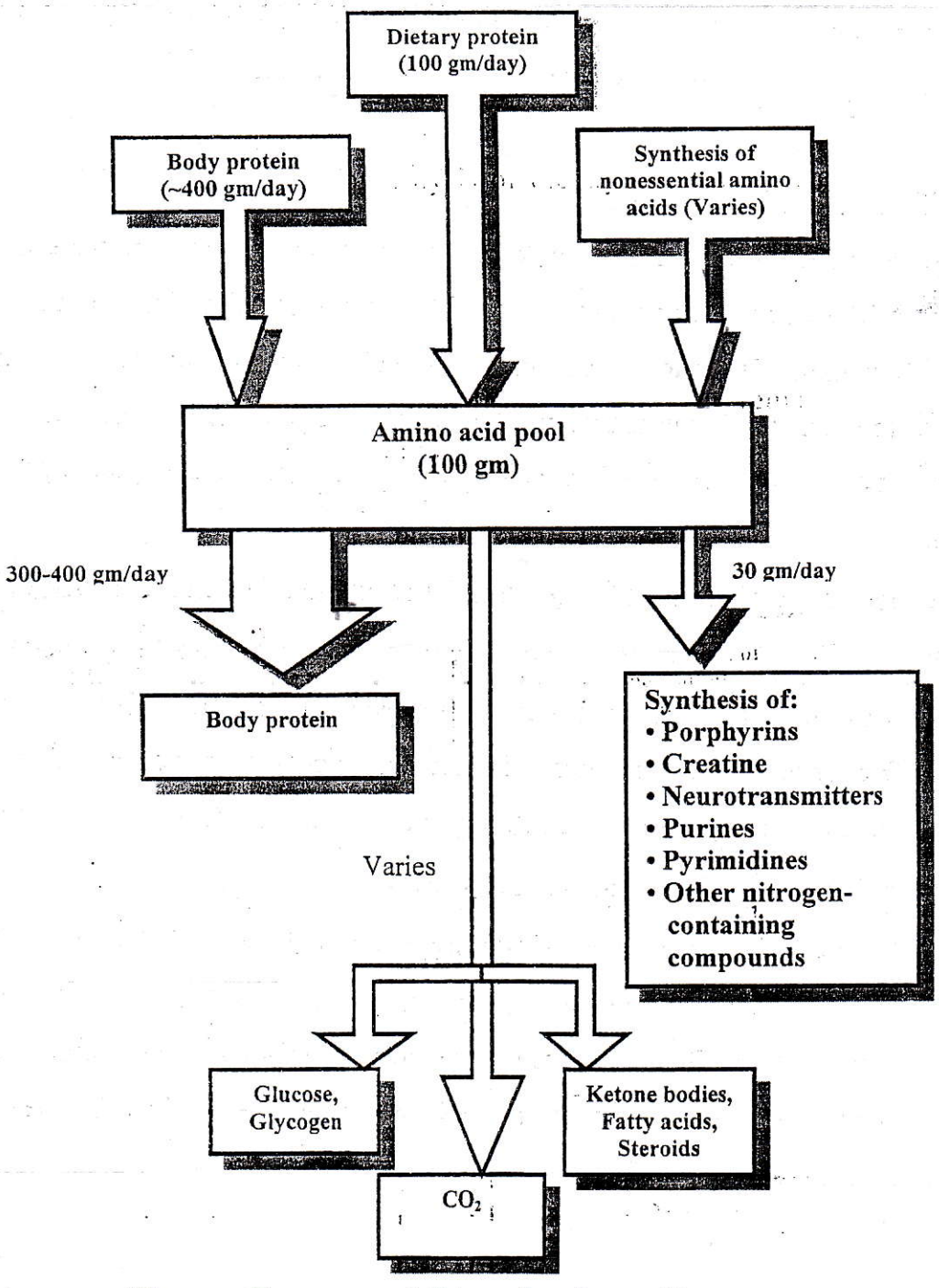


Figure : Sources and fates of amino acids.

Protein turnover:

Most proteins in the body are constantly being synthesized and then degraded. In healthy adults, the total amount of protein in the body remains constant, because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and resynthesis of 300 to 400 gm of body protein each day.

In contrast to carbohydrates and triacylglycerols whose major function is to provide energy, the primary role of amino acids to serve as building blocks in biosynthetic reactions, particularly the synthesis of tissue protein. Protein is used secondarily as a fuel.

Consequences of diets low in protein: If the diet does not provide adequate amounts of protein, a deficiency of essential amino acids required for the synthesis of body protein occurs. This results in the net breakdown of tissue protein that can lead to clinical symptoms of protein deficiency, such as those described for **kwashiorkor**.

Consequences of diets ~~low~~ high in protein: There is no storage form for amino acids analogous to that for lipid (triacylglycerol) or carbohydrate (glycogen). Therefore, if the diet contains excess protein, providing more amino acids than can be rapidly incorporated into protein or other nitrogen-containing molecules, the excess amino acids are metabolized, with their carbon skeletons being oxidized or converted to glucose or to fat, and their amino groups converted to ammonia.

Transport of amino acids into cells:

Active transport systems are required for movement of amino acids from the extracellular space into cells. At least seven different transport systems are known that have overlapping specificity for different amino acids. One transport system is responsible for reabsorption in kidney tubules of the amino acids cysteine, ornithine, arginine, and lysine. In the inherited disorder **cystinuria**, this carrier system is defective, resulting in the appearance of all four amino acids in the urine. Cystinuria occurs at a frequency of 1 to 7000 individuals, making it one of the most common inherited diseases and the most common genetic error of amino acid transport. The

disease expresses itself clinically by the precipitation of cystine to form kidney stones (calculi), which can block the urinary tract.

Removal of nitrogen from amino acids:

The first step in the catabolism of all amino acids involves the removal of the α-amino group. Once removed, this nitrogen can be incorporated into other compounds or excreted.

A) Transamination (the funneling of amino groups to glutamate):

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate. The products are an α-keto acid (derived from the original amino acid) and glutamate. Glutamate produced by transamination can be oxidatively deaminated, or can be used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino acid groups from one carbon skeleton to another is catalyzed by a family of enzymes called aminotransferases (formerly called transaminases). All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism.

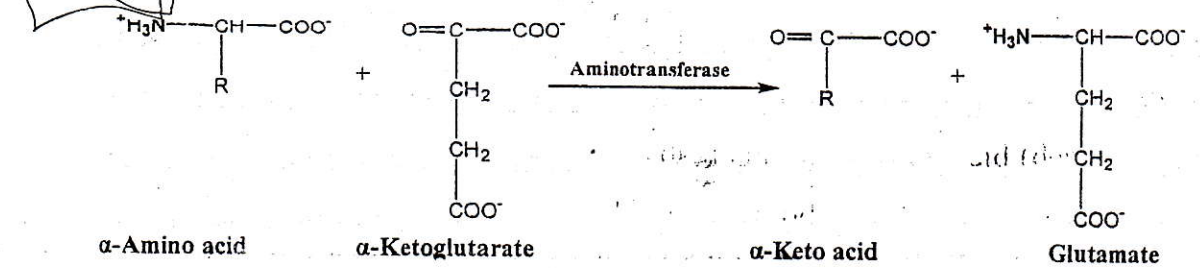


Figure : Aminotransferase reaction (transamination) using α-ketoglutarate as amino acceptor.

1. Substrate specificity of aminotransferases: Each aminotransferase is specific for one or at least a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate.

a. ***Alanine aminotransferase*** **(ALT),** also called as glutamate:pyruvate transaminase (GPT), is present in many tissues. The enzyme catalyzes the transfer of the amino

group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate.

Alanine + α-Ketoglutarate $\xrightarrow{\text{Alanine aminotransferase}}$ Pyruvate + Glutamate

Figure : Reaction catalyzed by alanine aminotransferase.

b. ***Aspartate aminotansferase*** **(AST),** also called glutamate:oxaloacetate transaminase (GOT), is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, aspartate aminotransferase transfer amino groups from glutamate to oxaloacetate, forming aspartate, which itself used as a source of nitrogen in the urea cycle.

2. Mechanism of action of aminotransferases: All aminotransferases require the coenzyme **pyridoxal phosphate**, which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate **pyridoxamine phosphate**. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid and regenerates the original aldehyde form of the coenzyme.

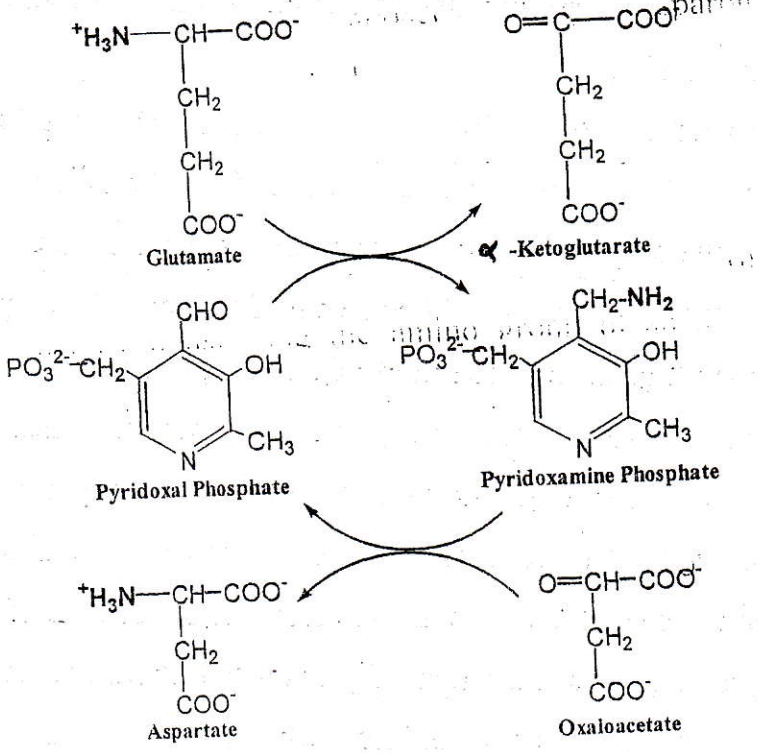


Figure : Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the aspartate aminotransferase reaction.

3. Diagnostic value of plasma aminotransferases: Aminotransferase are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferases in the plasma indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lyses, resulting in release of intracellular enzymes into the blood. Two aminotransferases when found in plasma are of particular diagnostic value: plasma aspartate aminotransferase (AST, formerly serum glutamate:oxaloacetate transaminase, SGOT) and plasma alanine aminotransferase (ALT, formerly serum glutamate:pyruvate transaminase, SGPT). Plasma AST and ALT are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis and prolonged circulatory collapse. Serial enzyme measurements are often useful in determining the course of liver damage.

B) Oxidative deamination:

In contrast to transamination reactions that transfer amino groups, oxidative deamination results in the liberation of the amino group as free ammonia. These reactions occur primarily in the liver and kidney and provide α-keto acids (which can enter the central pathway of energy metabolism) and ammonia (which is a source of nitrogen in urea synthesis).

As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by ***glutamate dehydrogenase***. Therefore, the sequential action of transamination (resulting in the collection of amino groups from other amino acids onto α-ketoglutarate to produce glutamate) and the subsequent oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia. Glutamate dehydrogenase is unusual in that it can use either NAD^+ or $NADP^+$ as a coenzyme. ATP and GTP are allosteric inhibitors of glutamate dehydrogenase, whereas GDP and ADP are activators of the enzyme. Thus, when energy levels are low in the cell,

amino acid degradation by glutamate dehydrogenase is high, providing α-ketoglutarate as a substrate for the TCA cycle.

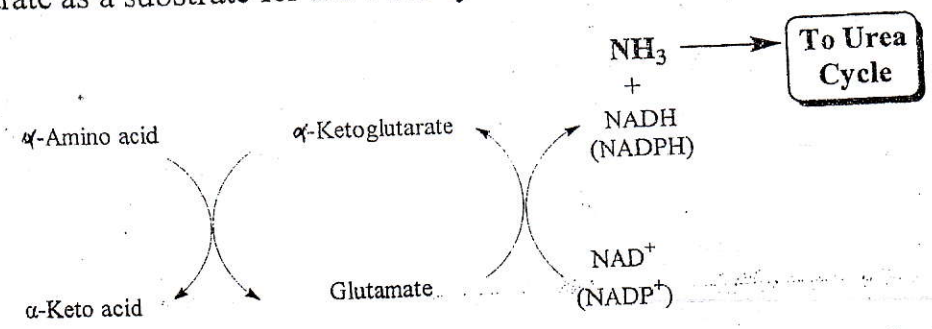


Figure : Combined actions of aminotransferase and glutamate dehydrogenase reactions.

UREA CYCLE:

Urea is the major disposal form of amino groups derived from amino acids, and accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free NH_3 and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by aspartate aminotransferase). The carbon and oxygen of urea are driven from CO_2. urea is produced by the liver and then is transported in the blood to the kidneys for excretion in the urine.

Reactions of the cycle:

The first two reactions leading to the synthesis of urea occur in the mitochondria, whereas the remaining cycle enzymes are located in the cytosol.

1. Formation of carbamoyl phosphate: Formation of carbamoyl phosphate by ***carbamoyl phosphate synthase I*** (the rate-limiting step) is driven by cleavage of two molecules of ATP. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate. Carbamoyl phosphate synthase I requires N-acetylglutamate for activity. [Note: A second enzyme, ***carbamoyl phosphate synthase II***, participates in the biosynthesis of pyrimidines. It does not require N-acetylglutamate, and occurs in the cytosol].

2. Formation of citrulline: Ornithine and citrulline are basic amino acids that participate in the urea cycle but are not incorporated into cellular proteins because there are no codons for these amino acids. Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the citric acid cycle. The release of the high-energy phosphate of carbamoyl phosphate as Pi drives the reaction in the forward direction. The reaction product, citrulline, is transported to the cytosol.

3. synthesis of argininosuccinate: Citrulline condenses with aspartate to form argininosuccinate. The formation of argininosuccinate is driven by the cleavage of ATP to AMP and PPi. This is the third and final molecule of ATP consumed in the formation of urea.

4. Cleavage of argininosuccinate: Argininosuccinate is cleaved to yield arginine and fumarate. The arginine formed by this reaction serves as the immediate precursor of urea. Fumarate produced in the urea cycle provides a link with several metabolic pathways: Fumarate is hydrated to malate, which is transported into the mitochondria and re-enters the TCA cycle. Alternatively, cytosolic malate can be oxidized to oxaloacetate, which cab be converted to aspartate or glucose.

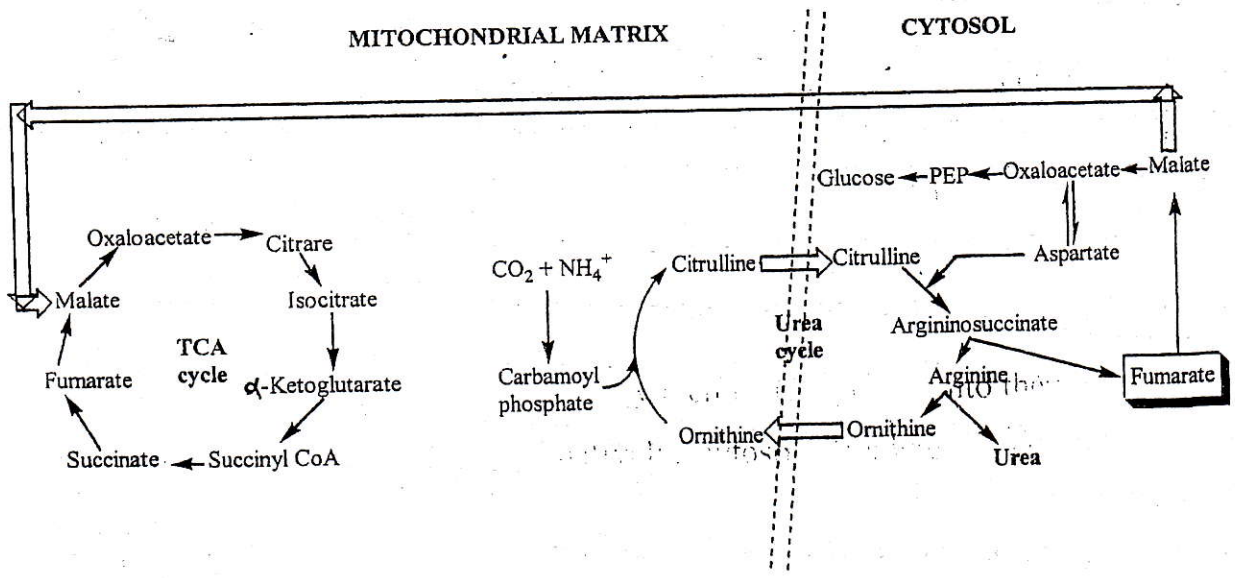


Figure : Fate of fumarate produced by the urea cycle.

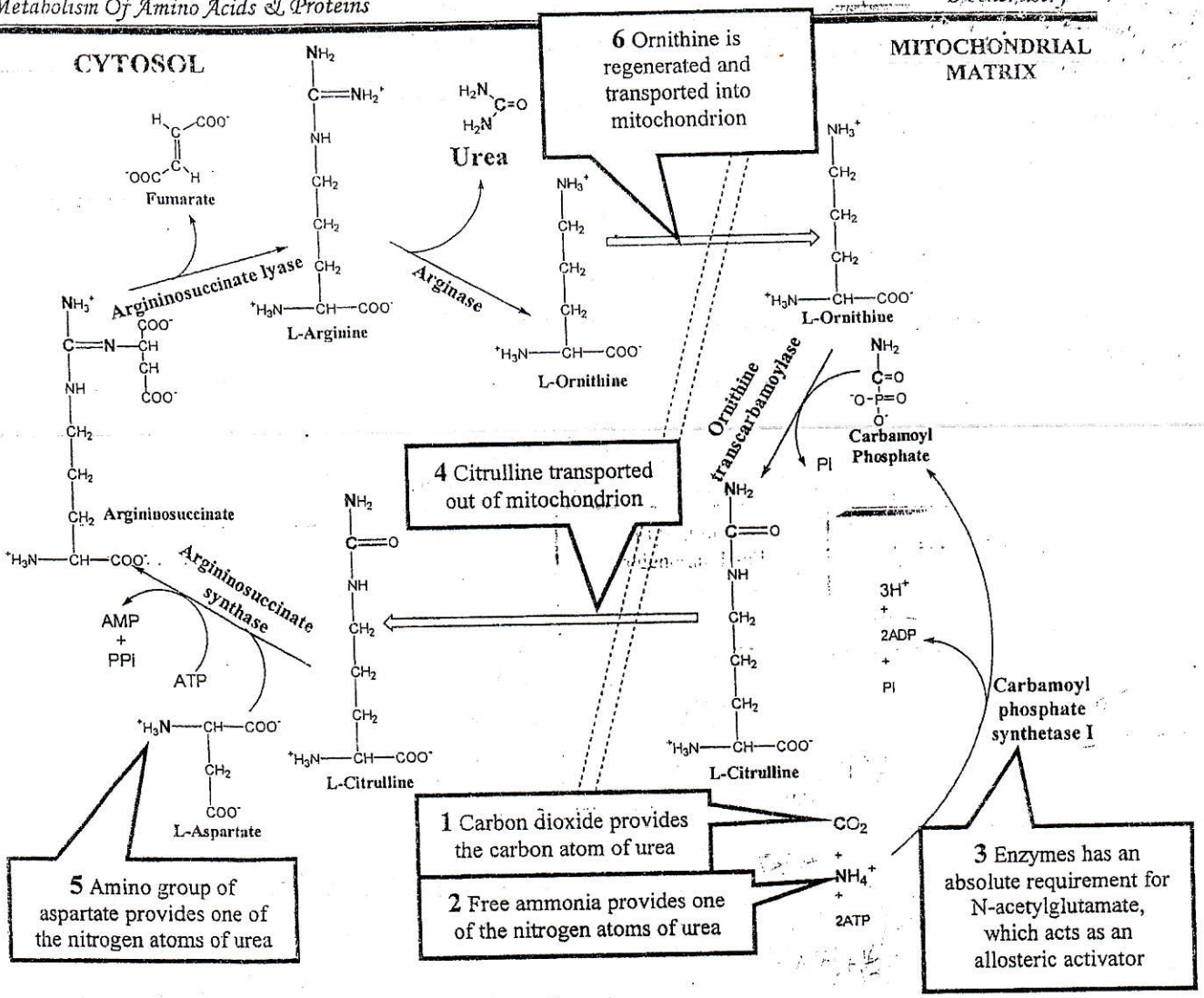


Figure : Reactions of urea cycle.

5. Cleavage of arginine to ornithine and urea: ***Arginase*** cleaves arginine to ornithine and urea. Arginase occurs almost exclusively in the liver. Thus, whereas other tissues can synthesize arginine, only the liver can cleave arginine and thereby synthesize urea.

6. Fate of urea: Urea diffuses from the liver and is transported in the blood to the kidneys, where it is filtered and excreted in the urine. A portion of the urea synthesized in the liver diffuses from the blood into the intestine and is cleaved to CO_2 and NH_3 by bacterial ***urease***. This ammonia is partly lost in the feces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The

intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the **hyperammonemia** often seen in these patients.

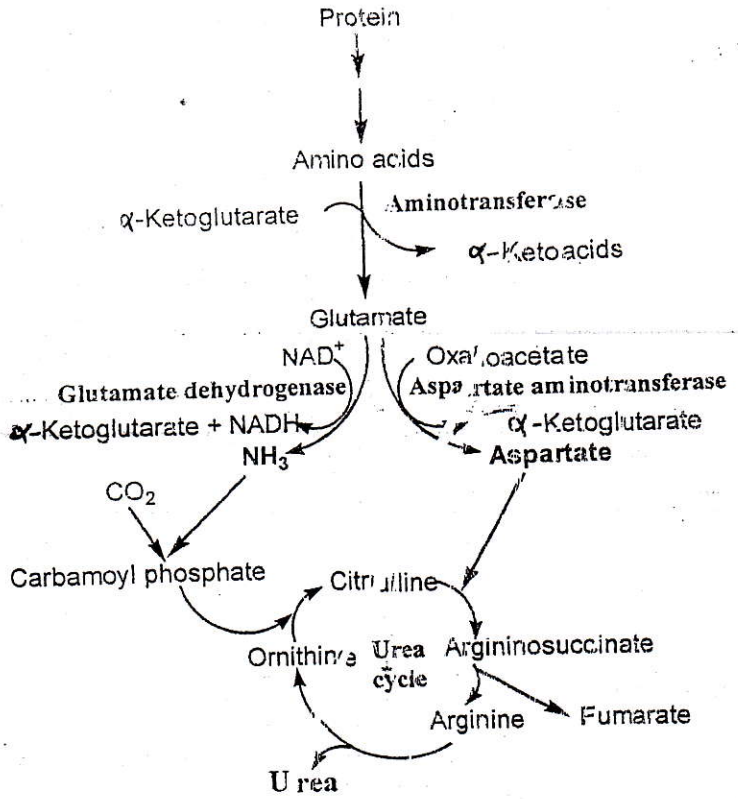


Figure : Flow of nitrogen from amino acids to urea.

METABOLISM OF AMMONIA:

Although ammonia is involved in the formation of urea in the liver, the level of ammonia in the blood must be kept low because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system. There must, therefore, be a metabolic mechanism by which nitrogen is moved from peripheral tissues to the liver for ultimate disposal as urea, at the same time maintaining low levels of circulating ammonia.

A. Sources of ammonia:

Ammonia is produced from the mechanism of a variety of compounds.

1. From amino acids: Many tissues, but particularly the liver, form ammonia from amino acids by the aminotransferase and glutamate dehydrogenase reactions.

2. From glutamine: The kidneys form ammonia from glutamine by the action of renal glutaminase. Most of this ammonia is excreted in the urine as NH_4^+, which is an

important mechanism for maintaining the body's acid-base balance. Ammonia is also obtained from the hydrolysis of glutamine by intestinal glutaminase.

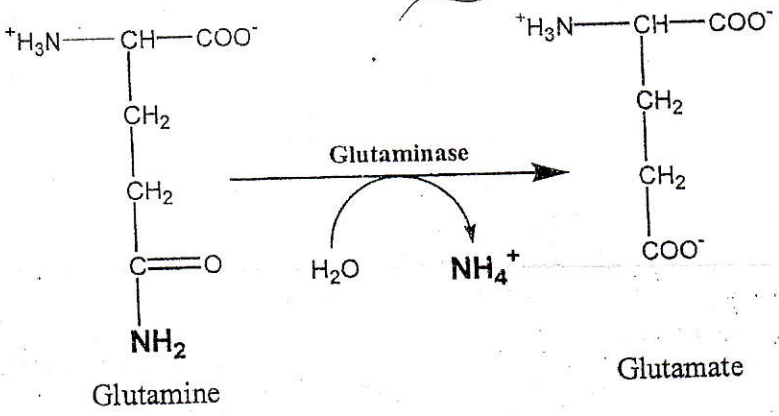


Figure : Hydrolysis of glutamine to form ammonia.

3. From bacterial action in the intestine: Ammonia is formed by the bacterial degradation of urea in the lumen of the intestine. Ammonia is absorbed from the intestine by way of the portal vein and is almost quantitatively removed by the liver by conversion to urea.

4. From amines: Amines obtained from the diet and monoamines that serve as hormones or neurotransmitters give rise to ammonia by the action of amine oxidase.

5. From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as ammonia.

B. Transport of ammonia in the circulation:

Although ammonia is constantly produced in the tissues, it is present at very low levels in blood. This is due to both the rapid removal of ammonia from the blood by the liver and the fact that many tissues, particularly muscle, release amino acid nitrogen in the form of glutamine and alanine, rather than as free ammonia.

1. Urea: Formation of urea in the liver is quantitatively the most important disposal route for ammonia. Urea travels in the blood from the liver to the kidneys, where it passes into the glomerular filtrate.

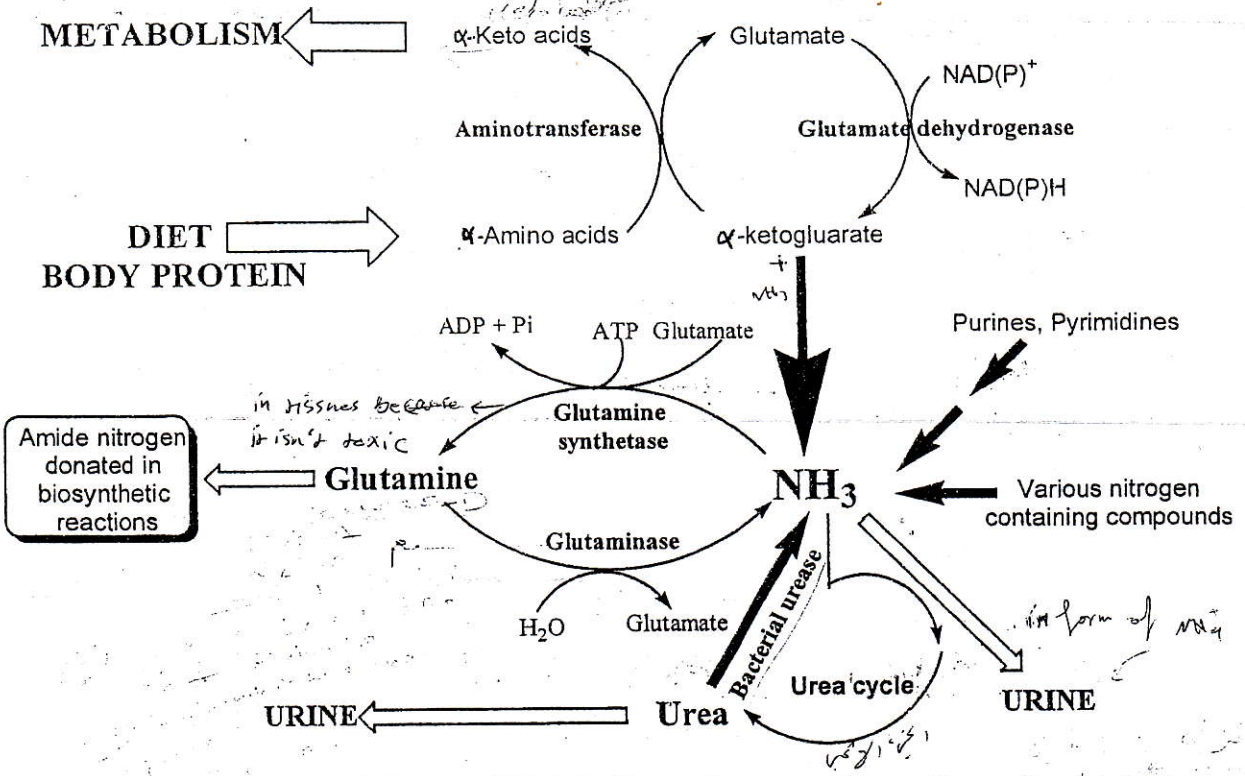


Figure : Metabolism of ammonia.

2. Glutamine: This amide of glutamic acid provides a nontoxic storage and transport form of ammonia. The formation of glutamine occurs primarily in the muscle and liver but also is important in the nervous system, where it is the major mechanism for the removal of ammonia in the brain. Glutamine is found in plasma at concentrations higher than other amino acids. Circulating glutamine is removed by the kidneys and deaminated by glutaminase.

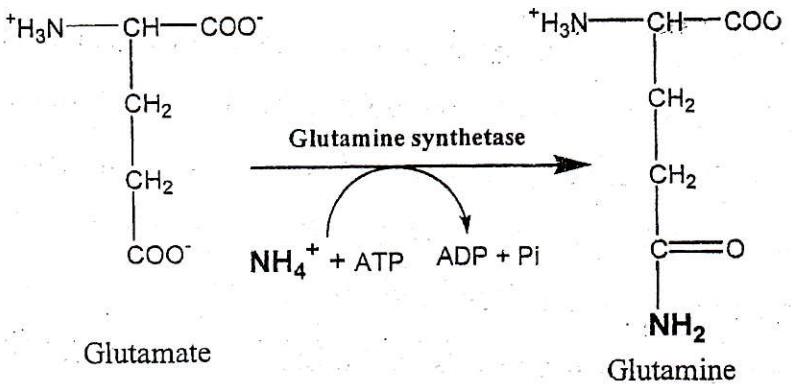


Figure : Synthesis of glutamine.

C. Hyperammonemia:

Elevated concentrations of ammonia in the blood cause the symptoms of **ammonia intoxication**, which include tremors, slurring of speech, and blurring of vision. At high concentrations ammonia can cause coma and death. The two major types of hyperammonemia are:

1. Acquired hyperammonemia: Cirrhosis of the liver caused by alcoholism, hepatitis, or biliary obstruction may result in formation of collateral circulation around the liver. As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver leading to elevated levels of circulating ammonia.

2. Hereditary hyperammonemia: Genetic deficiencies of each of the five enzymes of the urea cycle have been described, with an overall prevalence estimated to be 1 in 30000 live births. In each case the failure to synthesize urea leads to hyperammonemia during the first week following birth. All the inherited deficiencies of the urea cycle enzymes result in mental retardation.

Catabolism of the carbon skeletons of amino acids:

The catabolism of the 20 amino acids found in proteins involves the removal of α-amino groups followed by the breakdown of the resulting carbon skeletons. The catabolism of the carbon skeletons converges to form seven products: oxaloacetate, α-ketoglutarate, pyruvate, fumarate, acetyl CoA, acetoacetyl CoA, and succinyl CoA. These products enter the pathways of intermediary metabolism, resulting either in the synthesis of glucose or lipid, or in the production of energy through their oxidation to CO_2 and water by the TCA cycle.

Glucogenic and ketogenic amino acids:

Amino acids can be classified as **ketogenic** or **glucogenic** according to the nature of their metabolic end products. [Note: Amino acids can also be classified as **essential** or **nonessential** according to whether or not they can be synthesized in humans.

1. Glucogenic: Amino acids whose catabolism yields pyruvate or one of the intermediates of TCA cycle are termed glucogenic or glycogenic. These intermediates are substrates for gluconeogenesis and therefore can give rise to the net formation of glycogen in liver and muscle.

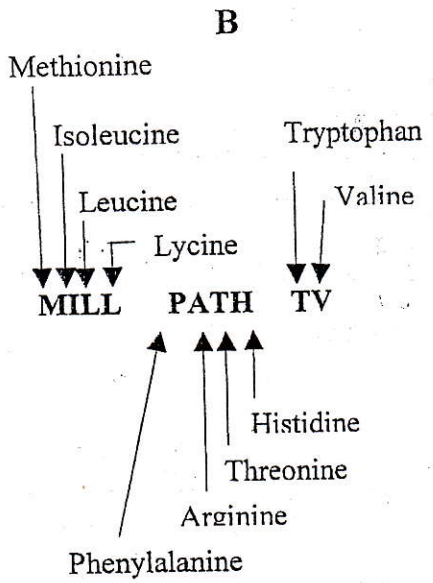
2. Ketogenic: Amino acids whose catabolism yields either acetoacetate or one of its precursors, acetyl CoA or acetoacetyl CoA, are termed ketogenic. Leucine and lysine are the only exclusively ketogenic amino acids found in proteins. These amino acids can not form glucose but are capable of yielding ketone bodies.

3. Glucogenic / Ketogenic: Amino acids of this group give rise to both glucose and ketone bodies.

A

	Glucogenic	Glucogenic & Ketogenic	Ketogenic
Nonessential	Alanine Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Essential	Arginine Histidine Methionine Threonine Valine	Isoleucine Phenylalanine Tryptophan	Leucine Lysine

B



not formed by the body

Figure : A. Classification of amino acids. B. The essential amino acids.

Metabolism of phenylalanine & tyrosine:

Phenylalanine is an essential amino acid, whereas, tyrosine is a nonessential amino acid. Tyrosine is formed from phenylalanine by ***phenylalanine hydroxylase.***

The reaction requires molecular oxygen and tetrahydrofolate. One atom of molecular oxygen becomes the hydroxyl group of tyrosine, and the other is reduced to water. Tyrosine is degraded to produce end products fumarate (glucogenic) and acetoacetate (ketogenic). Phenylalanine and tyrosine are therefore both glucogenic and ketogenic. Tyrosine is therefore nonessential only in the presence of adequate dietary phenylalanine. A genetic deficiency of phenylalanine hydroxylase results in the disease **phenylketonuria**.

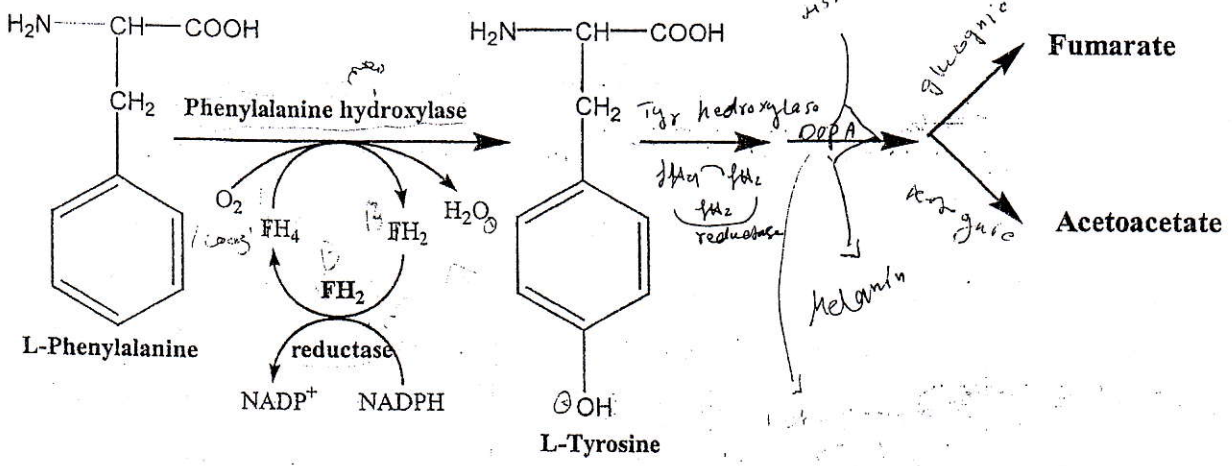


Figure : Degradation of phenylalanine.

METABOLIC DEFECTS IN AMINO ACID METABOLISM:

Inborn errors of metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes. The inherited defects may be expressed as a total loss of enzyme activity. Without treatment, the inherited defects of amino acid metabolism almost invariably result in mental retardation or other developmental abnormalities due to harmful accumulation of metabolities.

Hyperphenylalaninemias:

Phenylketonuria (PKU), caused by a deficiency of ***phenylalanine hydroxylase***, is the most clinically encountered inborn error of amino acid metabolism (prevalence 1:11000). [Note: PKU accounts for about half of the patients with elevated levels of plasma phenylalanine]. Hyperphenylalaninemia may also be caused by deficiencies in the enzymes that synthesize or reduce the coenzyme tetrahydrofolate (FH_4). It is frequently important to distinguish among these various forms of hyperphenylalaninemia, because their clinical management is different. For

example, a small fraction of PKU is due to the deficiency in the enzymes responsible for the synthesis of FH_4. these mutations prevent synthesis of FH_4 and indirectly rise phenylalanine concentrations, because phenylalanine hydroxylase requires FH_4 as a coenzyme. FH_4 is also required for tyrosine hydroxylase and tryptophan hydroxylase, which catalyze reactions leading to the synthesis of neurotransmitters such as serotonin and catecholamines. Simply restricting dietary phenylalanine does not reverse the CNS effects due to deficiencies in neurotransmitters. Replacement therapy with FH_4 improves the clinical outcome in these variant forms of hyperphenylalaninemia.

Characteristics of PKU:

1. Elevated phenylalanine: Phenylalanine is present in elevated concentrations in tissue, plasma, and urine. Phenyllactate, and phenylpyruvate, which are not normally produced in significant amounts in the presence of functional phenylalanine hydroxylase, are also elevated in PKU. Phenylacetate is conjugated with glutamine (in liver) and excreted in the urine as phenylacetylglutamine (responsible for 'mousy' odor of urine).

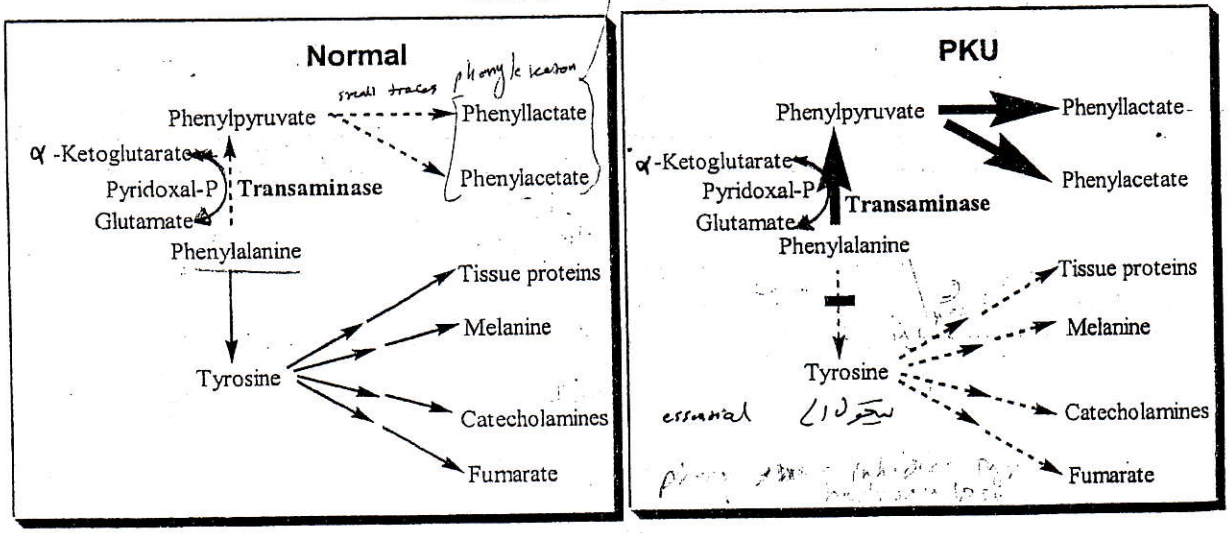


Figure : Pathways of phenylalanine metabolism in normal individuals and in patients with phenylketonuria (PKU).

2. CNS symptoms: Mental retardation, failure to walk or talk, seizures, tremor, microcephaly, and failure to grow are characteristic findings in PKU. The patients

with untreated PKU typically shows symptoms of mental retardation by the age of one year.

3. Hypopigmentation: Patients with PKU show a deficiency of pigmentation (fair hair, light skin colour, and blue eyes). The hydroxylation of tyrosine by ***tyrosinase*** (***tyrosine hydroxylase***) which is the first step in formation of the pigment **melanin**, is competitively inhibited by the high levels of phenylalanine present in PKU.

Neonatal diagnosis of PKU:

PKU is detected by neonatal screening for elevated blood levels of phenylalanine using the Guthrie test. However, the PKU infant frequently has normal blood levels of phenylalanine at birth because the mother clears increased blood phenylalanine in her affected fetus through the placenta. Thus, tests performed at birth may show false negative results. Normal levels of phenylalanine may persist until the newborn is exposed to at least 24 hours of protein feeding. Blood levels of phenylalanine should be determined on a second blood sample obtained after the infant has ingested protein. Normally, feeding breast milk or formula for 48 hours is sufficient to raise the baby's blood phenylalanine to levels that can be used for diagnosis.

Treatment of PKU:

Blood phenylalanine is maintained in the normal range by feeding synthetic amino acid preparations low in phenylalanine, supplemented with some natural foods selected for their low phenylalanine content. The amount is adjusted according to the tolerance of the individual as measured by blood phenylalanine levels. The earlier treatment is started, the more completely neurologic damage can be prevented. Treatment should not be delayed beyond the first month of life. Because phenylalanine is an essential amino acid, overzealous treatment that result in blood phenylalanine levels below normal should be avoided because it can lead to poor growth and neurologic symptoms. In patients with PKU, tyrosine can not be synthesized from phenylalanine and, hence, becomes essential and must be supplied in the diet.

Phenylalanine when accumulates in the body it inhibits the conversion of pyruvate to acetyl CoA. Thus depriving the cells of energy via the common pathway. This is the most important in brain, which gets its energy from the utilization of glucose. The result is mental retardation.

Conversion of amino acids to specialized products:

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen-containing compounds that have important physiologic functions. These functions include porphyrins, neurotransmitters, hormones, purines, and pyrimidines.

1. Creatine:

Creatine phosphate, the phosphorylated derivative of creatine found in muscle, is a high-energy compound that can reversibly donate a phosphate group to ADP to form ATP. the reaction, catalyzed by ***creatine kinase***, provides a small but rapidly mobilized reserve of high-energy phosphates that can be used to maintain the intracellular level of ATP during the first few minutes of intense muscular contraction.

Synthesis of creatine:

Creatine is synthesized from glycine and guanidine group of arginine, plus a methyl group from S-adenosylmethionine Figure . Creatine is reversibly phosphorylated to creatine phosphate by ***creatine kinase*** using ATP as phosphate donor. Creatine phosphate functions as a store of high-energy phosphate in muscle. [Note: The amount of creatine phosphate is proportional to the muscle mass].

Degradation of creatine:

Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine. The amount of creatinine excreted from the body is proportional to the total creatine phosphate content of the body, and thus can be used to estimate muscle mass. When muscle mass decreases for any reason (for example, from paralysis or muscular dystrophy), the creatinine content of the urine falls. In addition any rise in blood creatinine is a

sensitive indicator of kidney malfunction, because creatinine is normally rapidly removed from the body and excreted. A typical adult male excretes about 15 mmol of creatinine /day. Urine of normally healthy adult male contains creatinine but no creatine. Excretion of creatine in urine is called as **creatinuria**. This may be occur in pregnancy, lack of carbohydrate in diet, diabetes mellitus, starvation, and muscular dystrophies.

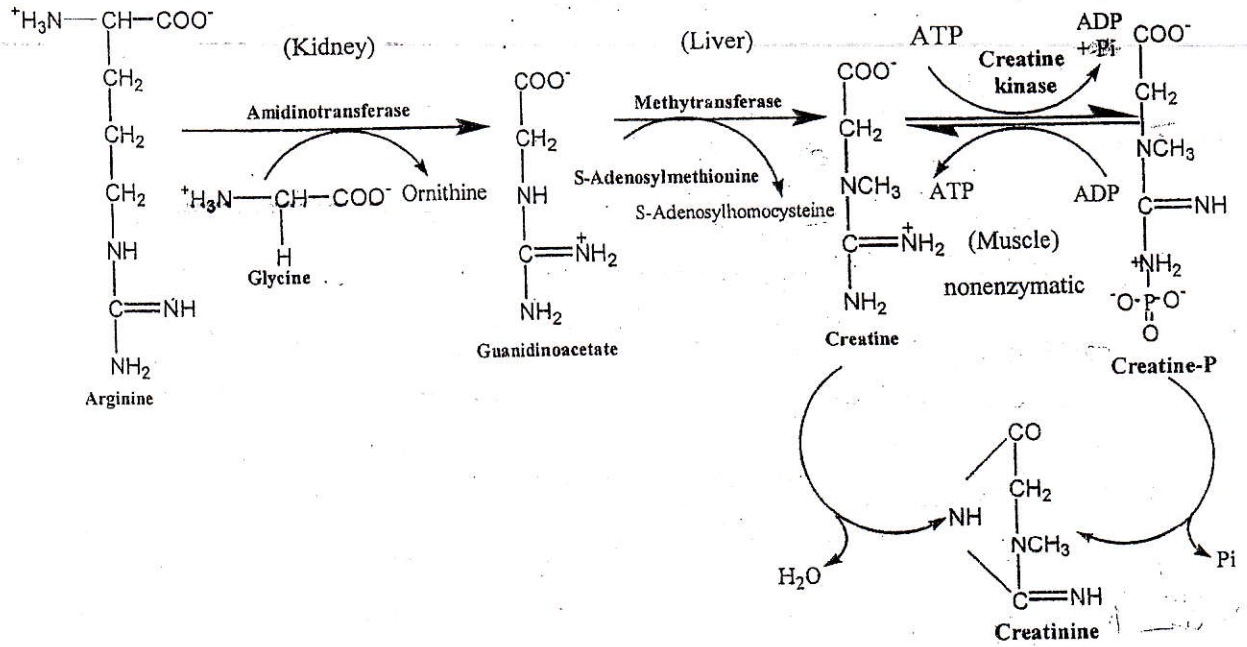


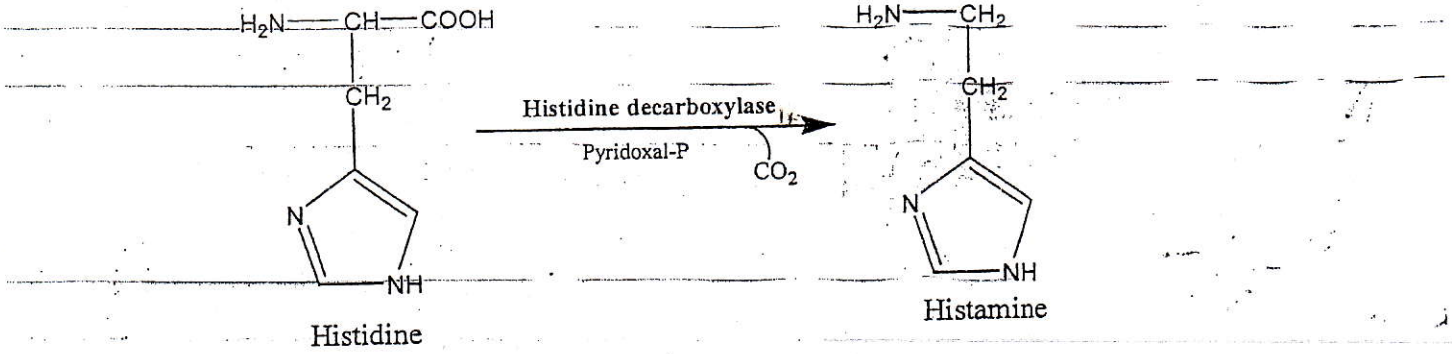
Figure : Synthesis of creatine.

2. Histamine:

Histamine is a chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion. Histamine, a powerful vasodilator (therefore depresses blood pressure), is formed by decarboxylation of histidine (Figure). It is secreted by mast cells (chief source of histamine in the tissues), also produced by gastric mucosal cells, and basophiles (chief source of histamine in the circulating cells) as a result of allergic reactions or trauma.

There are 2-kinds of receptors for histamine. One receptor, H_1 (in the respiratory tract), can be blocked by classic antihistamines such as diphenhydramine (Benadryl) and promethazine (Phenergan). Therefore, the anaphylactic (allergic) reactions can be minimized. The other receptor, H_2 (mainly in the stomach and affect

the secretion of HCl), can be blocked by ranitidine (Zantac) and Cimitidine, therefore reduce acid secretion and thus is an effective drugs for ulcer patients.



mast cell/basophilic cells gastric cells.

Figure : Synthesis of histamine.

3. γ-Aminobutyric acid (GABA):

GABA is formed in the tissues of the central nervous system, principally in the gray matter, by the decarboxylation of L-glutamic acid (Figure). It is released at the axonterminals and acts as inhibitory neurotransmitter (reduce neurotransmission).

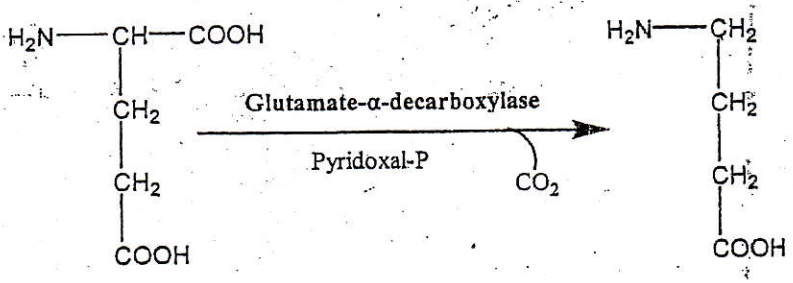


Figure : Synthesis of GABA.

4. Catecholamines:

Dopamine, norepinephrine, and epinephrine (adrenalin) are biologically active amines that collectively are termed catecholamines. Dopamine and norepinephrine function as neurotransmitters in the brain and autonomic nervous system. Norepinephrin and epinephrine are also synthesized in the adrenal medulla.

Functions:

Outside the nervous system, norepinephrine and its methylated derivative epinephrine act as regulators of carbohydrate and lipid metabolism. Norepinephrine and epinephrine are released from storage vesicles in the adrenal medulla in response to fright, exercise, cold, and low levels of blood glucose. Norepinephrine and epinephrine increase the degradation of triacylglycerol and glycogen as well as

increase the output of the heart and blood pressure. These effects are part of a coordinated response to prepare the individual for emergencies and are often called the "fight or flight' reactions.

Synthesis of catecholamines:

The catecholamines are synthesized from tyrosine (Figure). Tyrosine is first hydroxylated to form 3,4-dihydroxyphenylalanine (dopa). [Note: This reaction is the rate-limiting step of the pathway; the enzyme is abundant in the central nervous system, the sympathetic ganglia, and the adrenal medulla]. Dopa is decarboxylated to form dopamine, which is hydroxylated by a copper-containing ***hydroxylase*** to yield norepinephrine. Epinephrine is formed from norepinephrine by an N-methylation reaction using S-adenosylmethionine as methyl donor.

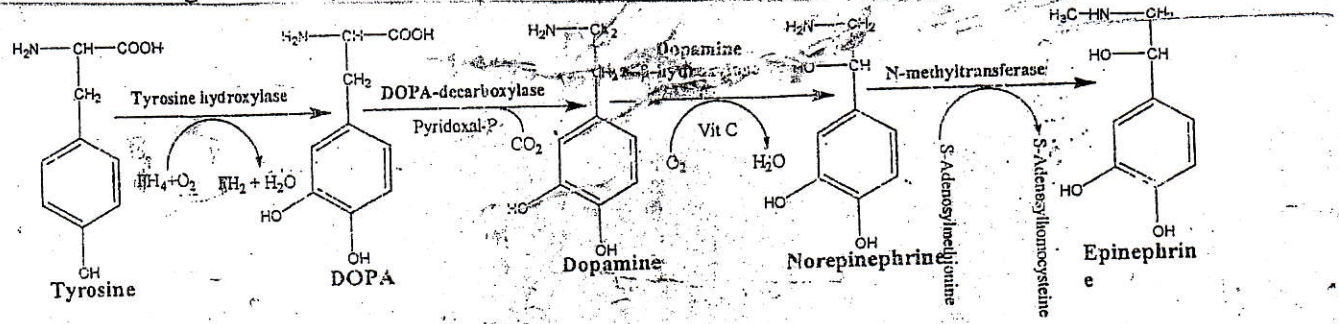


Figure : Synthesis of catecholamines.

5. Melanin:

Melanin is a pigment that occurs in a number of tissues in the body, particularly in the eye, hair, and skin. In the epidermis, the pigment-forming cells are called melanocytes. Here, melanin is synthesized to protect underlying cells from the harmful effects of sunlight. The first step in melanin formation from tyrosine is a hydroxylation reaction to form dopa. The subsequent reactions leading to the formation of black to brown and yellow to reddish-brown pigments. Biosynthesis of melanin is complex and not fully understood.